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A2

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A61K 31/405, A61K 37/02

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The application is published incomplete as filed (Article 93 (2) EPC). The points in the description at which the omissions obviously occur have been left blank.

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(54) Polyamines and polypeptides useful as antagonists of excitatory amino acid neurotransmitters and/or as blockers of calcium channels.

(57) This invention relates to certain polyamines and polypeptides found to be present in the venom of the *Agelenopsis aperta* spider. The polyamines of this invention and the salts thereof antagonize excitatory amino acid neurotransmitters, which neurotransmitters affect cells of various organisms and are useful in antagonizing said neurotransmitters, per se, in the treatment of excitatory amino acid neurotransmitter mediated diseases and conditions and in the control of invertebrate pests. The polypeptides of this invention and one of said polyamines and the salts thereof block calcium channels in cells of various organisms and are useful in blocking said calcium channels in cells, per se, in the treatment of calcium channel mediated diseases and conditions and in the control of invertebrate pests. This invention also relates to compositions comprising said polyamines, polypeptides and salts thereof.

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**POLYAMINES AND POLYPEPTIDES USEFUL AS ANTAGONISTS OF EXCITATORY AMINO ACID  
NEUROTRANSMITTERS AND/OR AS BLOCKERS OF CALCIUM CHANNELS**

This invention relates to certain polyamines and polypeptides found to be present in the venom of the Agelenopsis aperta spider. The polyamines and the pharmaceutically-acceptable salts thereof antagonize excitatory amino acid neurotransmitters, which neurotransmitters affect cells including neuronal cells of a variety of organisms including invertebrates and vertebrates. The polypeptides and one of said polyamines and the pharmaceutically-acceptable salts thereof block calcium channels in cells including neuronal and muscle cells of various organisms including invertebrates and vertebrates. This invention also relates to the use of such polyamines and their salts in antagonizing excitatory amino acid neurotransmitters, which neurotransmitters affect cells such as cells in the nervous system of an organism, per se, in the treatment of excitatory amino acid neurotransmitter mediated diseases and conditions in a mammal and control of invertebrate pests, and to compositions comprising said polyamines and salts thereof. Further, this invention relates to the use of said polypeptides and one of said polyamines and their salts in blocking calcium channels in cells such as cells in the nervous and muscular system of an organism, per se, in the treatment of calcium channel mediated diseases and conditions in a mammal, and in the control of invertebrate pests, and to compositions comprising said polypeptides, polyamine and salts thereof.

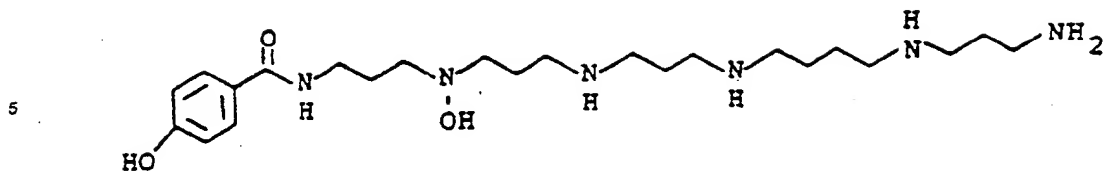
It has been reported that the venom of the spider Agelenopsis aperta contains at least two toxins which affect calcium currents. Jackson, H., et al., Soc. Neu. Sci. Abstr. 12:1078 (1987). Those authors disclose a toxin, referred to therein as AG2, which has a molecular weight of less than 1,000 daltons and appears to suppress calcium currents in a broad range of tissues. Further, Jackson, H., et al., Soc. Neu. Sci. Abstr. 12:730 (1986) report another toxin from Agelenopsis aperta comprising a component of about 6,000 M.W. That toxin is reported to effect presynaptic blockade of transmission and it has been suggested that the toxin blocks calcium channels associated with the release of neurotransmitter.

Compounds which are excitatory amino acid neurotransmitter antagonists have a variety of utilities. Excitatory amino acid neurotransmitter antagonists can find clinical application in the treatment of such conditions as seizure, stroke, cerebral ischemia, neuronal degeneration disorders such as Alzheimer's disease and epilepsy and as psychotherapeutants, among others. See Excitatory Amino Acids in Health and Disease, D. Lodge, Ed., John Wiley and Sons Ltd., New York, NY 1988, the teachings of which are incorporated herein by reference. Further, such compounds are useful in the study of the physiology of cells such as neuronal cells and in the control of invertebrate pests.

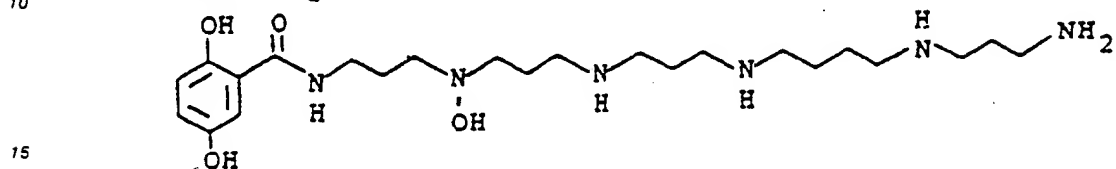
Compounds which are calcium antagonists have a variety of utilities. Calcium antagonists can find clinical application in the treatment of such conditions as angina, hypertension, cardiomyopathies, supraventricular arrhythmias, esophageal achalasia, premature labor and Raynaud's disease among others. See W. G. Naylor, Calcium Antagonists, Academic Press, Harcourt Brace Jovanovich Publishers, New York, NY 1988, the teachings of which are incorporated herein by reference. Further, such compounds are useful in the study of the physiology of cells such as neuronal and muscle cells and in the control of invertebrate pests.

This invention concerns polyamines and polypeptides found to be present in the venom of the Agelenopsis aperta spider. The polyamines of this invention and the fractions in which they are present according to this invention are as follows.

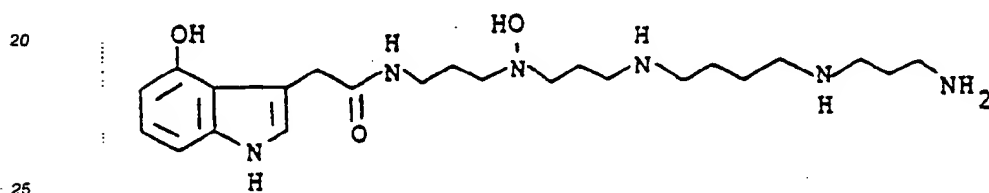
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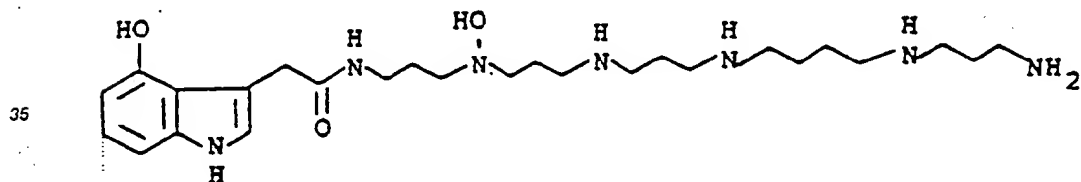
Fraction A<sub>1</sub> (AGEL 468):



Fraction A<sub>2</sub> (AGEL 448):



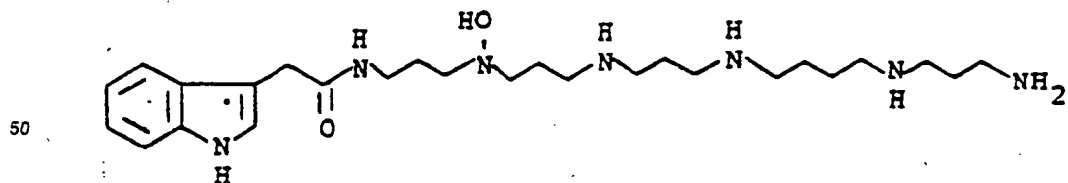
Fraction B<sub>1</sub> (AGEL 505):



Fraction B<sub>2</sub> (AGEL 504):

FAB MS: high resolution: 505.3861 calc. for  
C<sub>27</sub>H<sub>48</sub>N<sub>6</sub>O<sub>3</sub>.

Fraction E (AGEL 489):



55 The polypeptides of this invention and the fractions in which they are present according to this invention are as follows.

Fraction G:

An amino-terminal amino acid sequence comprising:

H<sub>2</sub>N-glu-lys-gly-leu-pro-glu-gly-ala-glu-cys-asp-gly-asn-glu-ser-asp-cys-lys-cys-ala-gly-gln-trp-ile-lys-cys-arg-cys-pro-trp-lys-trp-his-ile-thr-gly-glu-gly-pro-cys-thr-cys-glu-arg-gly-leu-lys-lys-thr-cys-ile-ser-lys-leu-ser-asp-pro-asn-arg-asn-glu-trp-leu-ser-; molecular weight for the entire polypeptide according to FAB MS: 7267.

5 Fraction H<sub>2</sub>:

An amino-terminal amino acid sequence comprising:

H<sub>2</sub>N-ala-lys-ala-leu-pro-pro-gly-ser-val-cys-asp-gly-asn-glu-ser-asp-cys-lys-cys-tyr-gly-lys-trp-his-lys-cys-arg-cys-pro-trp-lys-trp-his-phe-thr-gly-glu-gly-pro-cys-thr-cys-glu-lys-gly-met-lys-his-thr-cys-ile-thr-lys-leu-his-cys-pro-asn-lys-ala-glu-trp-gly-leu-asp-trp-; molecular weight for the entire polypeptide according to lon-

10 Spray MS: 7793.

Fraction I:

H<sub>2</sub>N-asp-cys-val-gly-glu-ser-gln-gln-cys-ala-asp-trp-a.1 a-gly-pro-his-cys-cys-asp-gly-tyr-tyr-cys-thr-cys-arg-tyr-phe-pro-lys-cys-ile-cys-val-asn-asn-asn-CONH<sub>2</sub>; FAB MS: 4158.

Fraction J:

15 H<sub>2</sub>N-asp-glu-pro-cys-ile-pro-leu-gly-lys-ser-cys-ser-trp-lys-ile-gly-thr-pro-tyr-cys-cys-pro-his-pro-asp-asp-ala-gly-arg-arg-thr-trp-cys-leu-val-asp-tyr-ser-arg-phe-val-thr-ile-cys-ser-gly-arg-lys-tyr-CONH<sub>2</sub>; molecular weight for the entire polypeptide according to FAB MS: 5506.

Fraction L<sub>1</sub>:

An amino-terminal amino acid sequence comprising:

20 H<sub>2</sub>N-ile-val-gly-gly-lys-thr-ala-lys-phe-gly-asp-tyr-pro-trp-met-val-ser-ile-gln-gln-lys-asn-lys-lys-gly-gly-phe-asp-; approximate molecular weight for the entire polypeptide of about 20,000.

Fraction L<sub>2</sub>:

A polypeptide obtained as described in Example 9.

Fraction M:

25 An amino-terminal amino acid sequence comprising:

H<sub>2</sub>N-glu-ala-thr-glu-ala-ala-lys-val-leu-ser-asn-leu-asp-glu-thr-val-asp-pro-; approximate molecular weight for the entire polypeptide of about 80,000.

The polyamines of this invention and the pharmaceutically-acceptable salts thereof antagonize excitatory amino acid neurotransmitters, which neurotransmitters affect cells. Thus, said polyamines are useful in antagonizing said neurotransmitters, per se. The polyamines of this invention are also useful in the control of invertebrate pests and in the treatment of diseases and conditions in a mammal mediated by excitatory amino acid neurotransmitters. Said polyamines are useful, also, as psychotherapeutants for a mammal.

35 The polyamine B<sub>1</sub>, above, and the polypeptides of this invention block calcium channels in cells. Thus, said polyamine B<sub>1</sub> and polypeptides are useful in blocking calcium channels in cells, per se. Said polyamine B<sub>1</sub> and said polypeptides are also useful in the control of invertebrate pests and in the treatment of diseases and conditions in a mammal mediated by calcium channel function in cells.

Also within the scope of this invention are polypeptides which have substantially the same amino acid sequence and substantially the same calcium channel blocking activity as the polypeptides described above.

40 This invention also concerns pharmaceutical compositions comprising said polyamines and polypeptides and methods of administering said polyamines and polypeptides.

Venom is obtained from the Agelenopsis aperta spider through the process of milking by electrical stimulation according to standard methods well known to those skilled in the art. It is preferred that the method employed is one which safeguards against contamination of the whole venom by abdominal regurgitant or hemolymph. Such methods are well known to those skilled in the art. The whole venom so obtained is stored in a frozen state at about -78° C. until used for purification as described below.

Purification of the constituents from the whole venom is accomplished by reverse phase high performance liquid chromatography (HPLC) on a variety of preparative and semi-preparative columns such as C-4 and C-18 Vydac® columns (Rainin Instrument Co. Inc., Mack Road, Woburn Massachusetts 01801). Peak detection is carried out monochromatically at 220-230 nm. Further analysis of the fractions can be accomplished with, for example, polychrome UV data collected with a Waters 990 diode array detector (Millipore Corporation, Waters Chromatography Division, 34 Maple Street, Milford, Massachusetts 01757). The fractions from the columns are collected by known methods such as through the use of an ISCO/FOXY fraction collector and an ISCO 2159 peak detector (ISCO, 4700 Superior, Lincoln, Nebraska 68504). The fractions are collected in appropriately sized vessels such as sterile polyethylene laboratory-ware. Concentration of the fractions is then accomplished by lyophilization from the eluant followed by lyophilization from water. Purity of the resulting constituent fractions then can be determined by chromatog-

raphic analysis using an analytical column with a gradient system which is more isocratic than the system used in the final purification of the fractions.

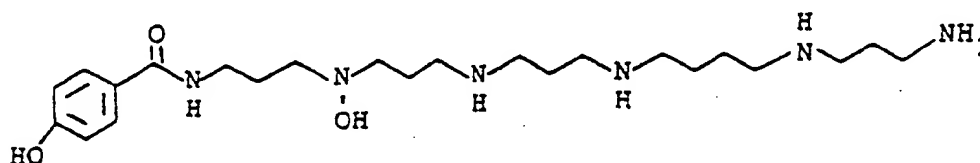
The structures comprised by the respective fractions are determined according to known analytical methods such as by mass spectrometry and nuclear magnetic resonance. The polypeptides of this invention are sequenced according to known methods. For example, S-pyridylethylation of cystine residues of the polypeptide under study can be performed in solution followed by amino acid sequencing of the polypeptide. One such procedure for S-pyridylethylation is as follows. About 1 to 10  $\mu$ g of polypeptide is dissolved or diluted in up to 50  $\mu$ l of a buffer prepared by mixing 1 part 1M TrisHCl, pH 8.5 containing 4 mM EDTA and 3 parts 8M guanidine<sup>+</sup>HCl. Then, 2.5  $\mu$ l of 10% aqueous 2-mercaptoethanol is added and the mixture is incubated at room temperature in the dark under argon for two hours. After incubation, 2  $\mu$ l of 4-vinylpyridine (fresh reagent stored under argon at -20<sup>o</sup> C.) is added and the mixture is incubated for another two hours at room temperature in the dark under argon. The mixture is then desalted, preferably by chromatography on a short, reverse phase column. The recovered alkylated polypeptide is then sequenced according to known methods.

In practicing this invention and employing the general procedure outlined above, it has been found that a suitable column for initial fractionation of the venom is a C-18 Vydac<sup>®</sup> 22 mm x 250 mm, 300 Å pore size, 10  $\mu$  particle size column. That column is eluted at a flow rate of 15 ml/min. using a linear gradient program of 95%  $\rightarrow$  80% A, 5%  $\rightarrow$  20% B [0  $\rightarrow$  30 min.] then 80%  $\rightarrow$  30% A, 20%  $\rightarrow$  70% B [30  $\rightarrow$  55 min.] where A is 0.1% aqueous trifluoroacetic acid (TFA) and B is acetonitrile. The fractions are collected as described above. Ten fractions so obtained, labeled A, B, E, G, H, I, J, K, L and M, were chosen for further analysis and/or purification. Additionally, it has been found that fractionation of the whole venom using a C-18 Vydac<sup>®</sup> 22 mm x 250 mm, 300 Å pore size, 10  $\mu$  particle size column which is eluted at a flow rate of 15 ml/min. using a linear gradient program of 5%  $\rightarrow$  10% B, 95%  $\rightarrow$  90% A [0  $\rightarrow$  30 min.] where A and B are as described above yields, inter alia, a fraction labelled A' herein. The elution times of the fractions are given below in Examples 1 and 2.

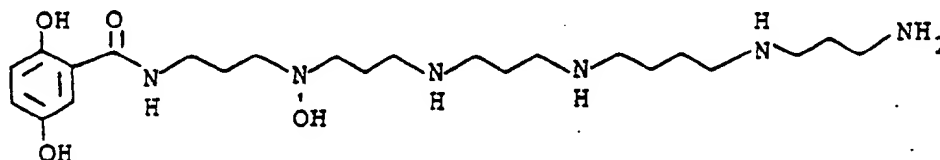
Fractions A, B, G, H, I/J and L are subjected to further purification using a variety of columns and gradient programs. The specifics for each subfractionation and the results thereof are given in Examples 3, 4, 6, 7, 8 and 10 below.

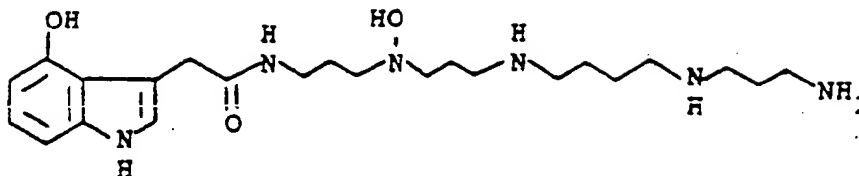
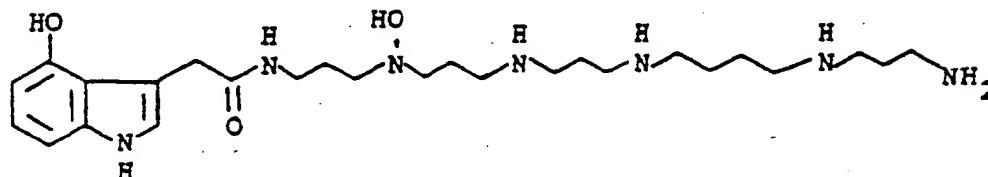
As shown in the following Examples, Fractions A', A<sub>1</sub>, A<sub>2</sub>, B<sub>1</sub>, B<sub>2</sub> and E comprise polyamine compounds. Those compounds and the fractions in which they are found are as follows.

#### Fraction A' (AGEL 452):



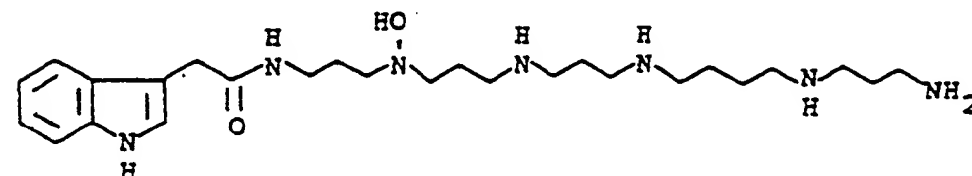
#### Fraction A<sub>1</sub> (AGEL 468):



Fraction A<sub>2</sub> (AGEL 448):Fraction B<sub>1</sub> (AGEL 505):Fraction B<sub>2</sub> (AGEL 504):

FAB MS: high resolution: 505.3861 calc. for  
C<sub>27</sub>H<sub>48</sub>N<sub>6</sub>O<sub>3</sub>.

## Fraction E (AGEL 489):



As shown in the following Examples, Fractions G, H<sub>2</sub>, I, J, L<sub>1</sub>, L<sub>2</sub> and M comprise polypeptides. Those polypeptides and the fractions in which they are found are as follows:

## Fraction G:

An amino-terminal amino acid sequence comprising: H<sub>2</sub>N-glu-lys-gly-leu-pro-glu-gly-ala-glu-cys-asp-gly-asn-glu-ser-asp-cys-lys-cys-ala-gly-gln-trp-ile-lys-cys-arg-cys-pro-trp-lys-trp-his-ile-thr gly-gly-gly-pro-cys-thr-cys-glu-arg-gly-leu-lys-lys-thr-cys-ile-ser-lys-leu-ser-asp-pro-asn-arg-asn-glu-trp-leu-ser-; molecular weight for the entire polypeptide according to FAB MS: 7267.

Fraction H<sub>2</sub>:

An amino-terminal amino acid sequence comprising:

H<sub>2</sub>N-ala-lys-ala-leu-pro-pro-gly-ser-val-cys-asp-gly-asn-glu-ser-asp-cys-lys-cys-tyr-gly-lys-trp-his-lys-cys-arg-cys-pro-trp-lys-trp-his-phe-thr-gly-glu-gly-pro-cys-thr-cys-glu-lys-gly-met-lys-his-thr-cys-ile-thr-lys-leu-his-cys-pro-asn-lys-ala-glu-trp-gly-leu-asp-trp-; molecular weight for the entire polypeptide according to Ion-Spray MS: 7793.

## Fraction I:

H<sub>2</sub>N-asp-cys-val-gly-glu-ser-gln-gln-cys-ala-asp-trp-ala-gly-pro-his-cys-cys-asp-gly-tyr-tyr-cys-thr-cys-arg-tyr-phe-pro-lys-cys-ile-cys-val-asn-asn-asn-CONH<sub>2</sub>; FAB MS: 4158.

## Fraction J:

H<sub>2</sub>N-asp-glu-pro-cys-ile-pro-leu-gly-lys-ser-cys-ser-trp-lys-ile-gly-thr-pro-tyr-cys-cys-pro-his-pro-asp-asp-ala-gly-arg-arg-thr-trp-cys-leu-val-asp-tyr-ser-arg-phe-val-thr-ile-cys-ser-gly-arg-lys-tyr-CONH<sub>2</sub>; FAB MS: 5506.

Fraction L<sub>1</sub>:

An amino-terminal amino acid sequence comprising:

H<sub>2</sub>N-ile-val-gly-gly-lys-thr-ala-lys-phe-gly-asp-tyr-pro-trp-met-val-ser-ile-gln-gln-lys-asn-lys-lys-gly-gly-phe-asp-; and an approximate molecular weight for the entire polypeptide of about 20,000.

Fraction L<sub>2</sub>:

A polypeptide obtained as described in Example 9.

Fraction M:

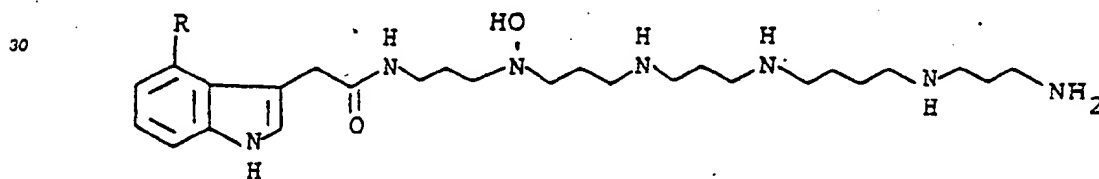
An amino-terminal amino acid sequence comprising:

H<sub>2</sub>N-glu-ala-thr-glu-ala-ala-lys-val-leu-ser-asn-leu-asp-glu-thr-val-asp-pro-; and an approximate molecular weight for the entire polypeptide of about 80,000.

Given the benefit of the disclosure herein with respect to the compounds present in the fractions of venom from *Agelenopsis aperta*, it is now possible to obtain said compounds by methods other than through isolation/purification from whole venom. All such methods are within the scope of this invention. For example, the polyamines which are comprised by fractions A', A<sub>1</sub>, A<sub>2</sub>, B<sub>1</sub>, B<sub>2</sub> and E can be made directly by synthetic methods. The polypeptides which are comprised by fractions G, H<sub>2</sub>, I, J, L<sub>1</sub>, L<sub>2</sub> and M and for which the entire amino acid sequence is described can be produced synthetically by *in vitro* protein synthesis according to well known methods. For example, such polypeptides can be synthesized using an ABI 430A solid phase peptide synthesizer (Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, California 94404) employing standard Merrifield chemistry or other solid phase chemistries well known to those skilled in the art. The polypeptides can also be produced using recombinant DNA techniques through the cloning of coding sequences for the polypeptides or portions thereof. Those polypeptides for which only part of the amino acid sequence is known can be cloned, for example, through the use of hybridization probes which take advantage of the now known amino acid sequence information. A combination of recombinant DNA techniques and *in vitro* protein synthesis can also be employed to produce the polypeptides of this invention.

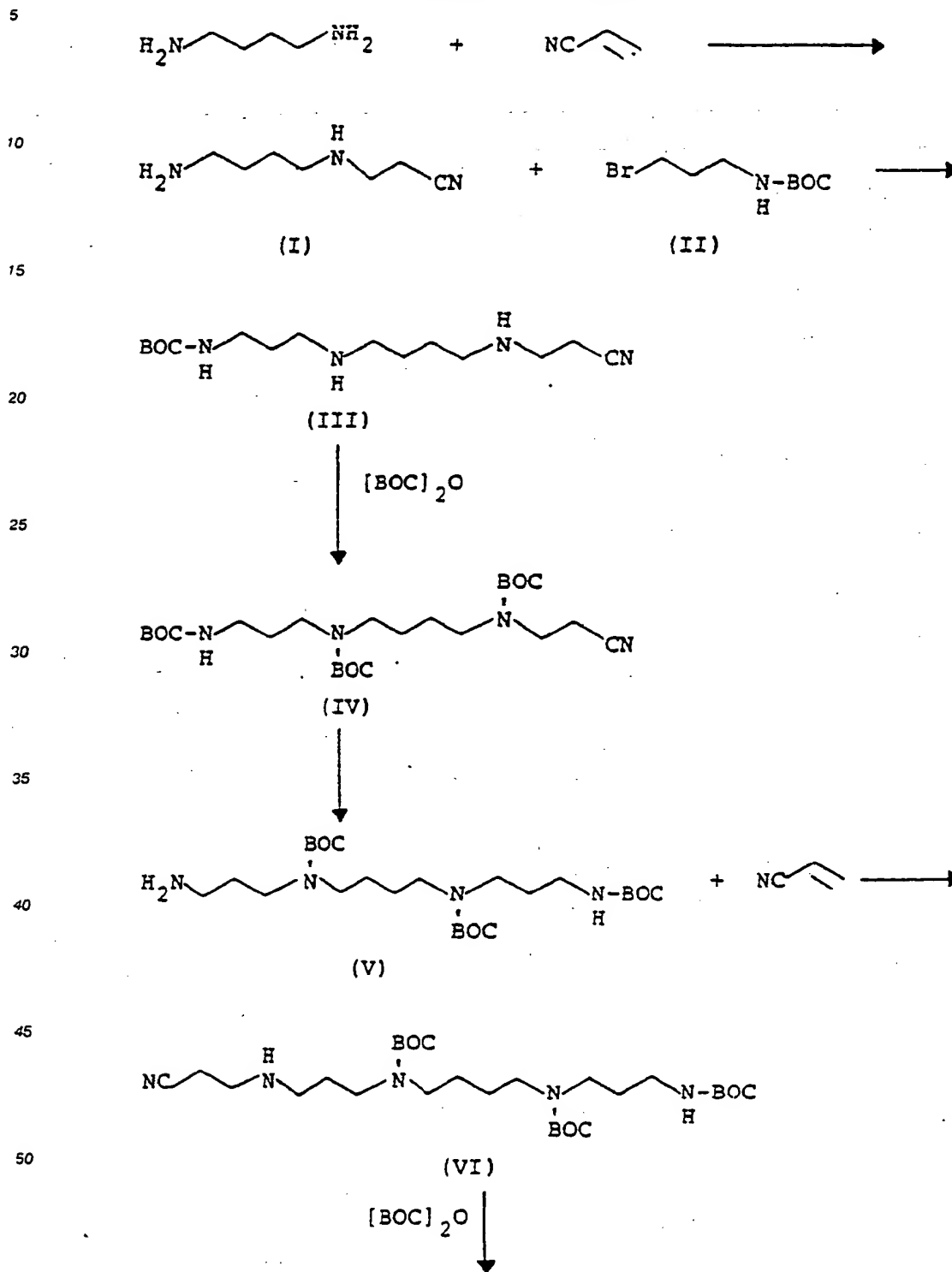
It is well known in the art that certain amino acid substitutions can be made in polypeptides which do not affect, or do not substantially affect, the function of said polypeptides. The exact substitutions which are possible vary from polypeptide to polypeptide. Determination of permissible substitutions is accomplished according to procedures well known to those skilled in the art. Thus, all polypeptides having substantially the same amino acid sequence and substantially the same calcium channel blocking activity are within the scope of this invention.

A synthetic scheme for production of certain polyamines of this invention of the formula

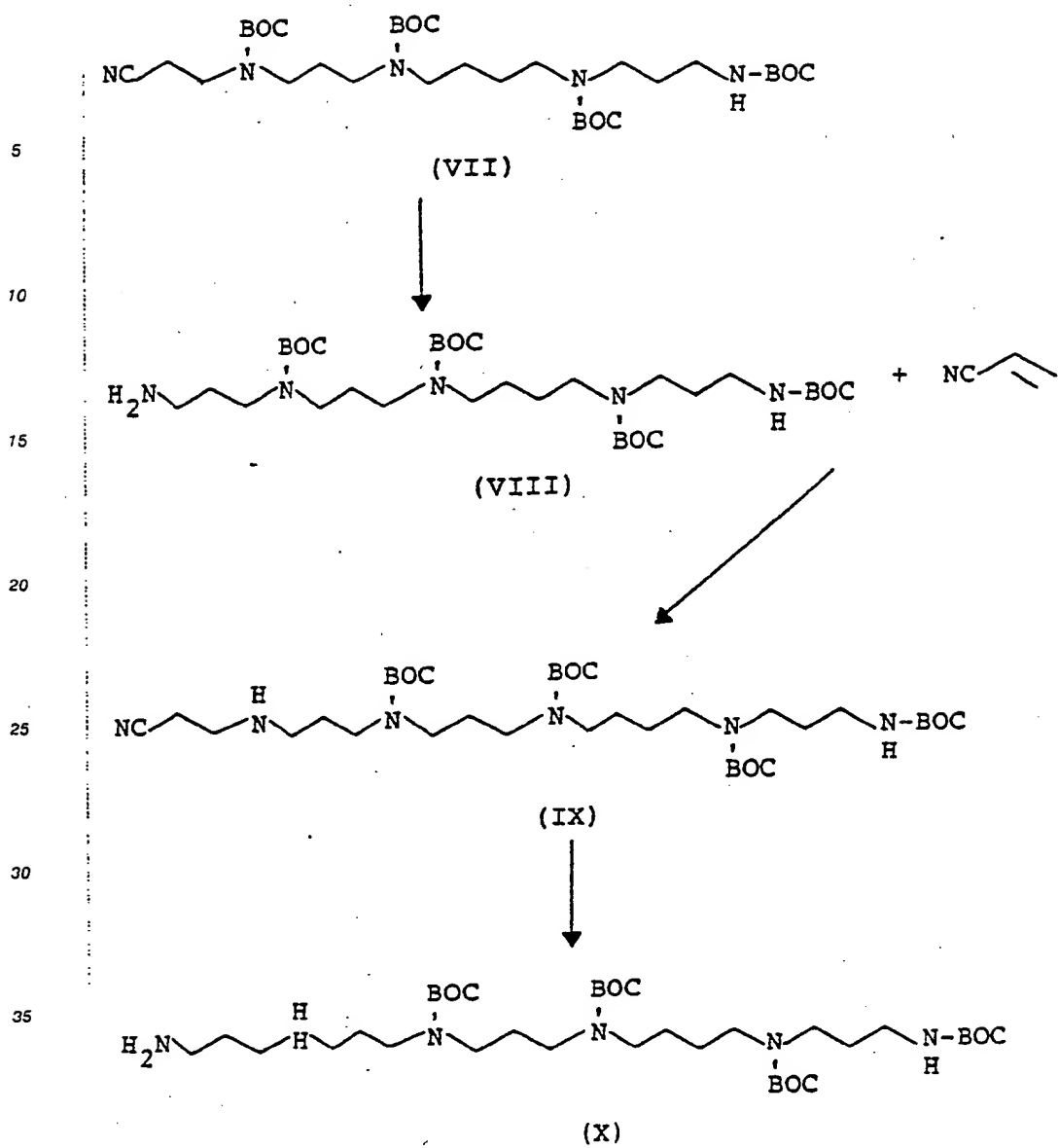


wherein R is H or OH is shown in Reaction Schemes A to D, below.

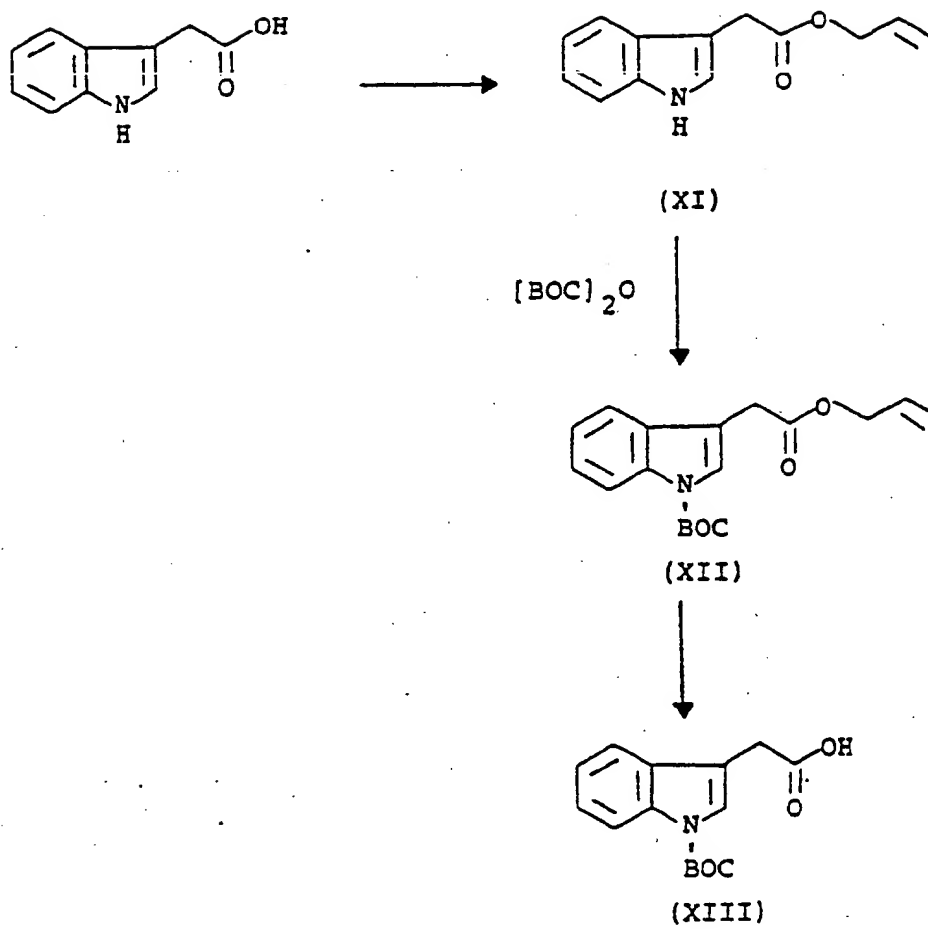
## REACTION SCHEME A



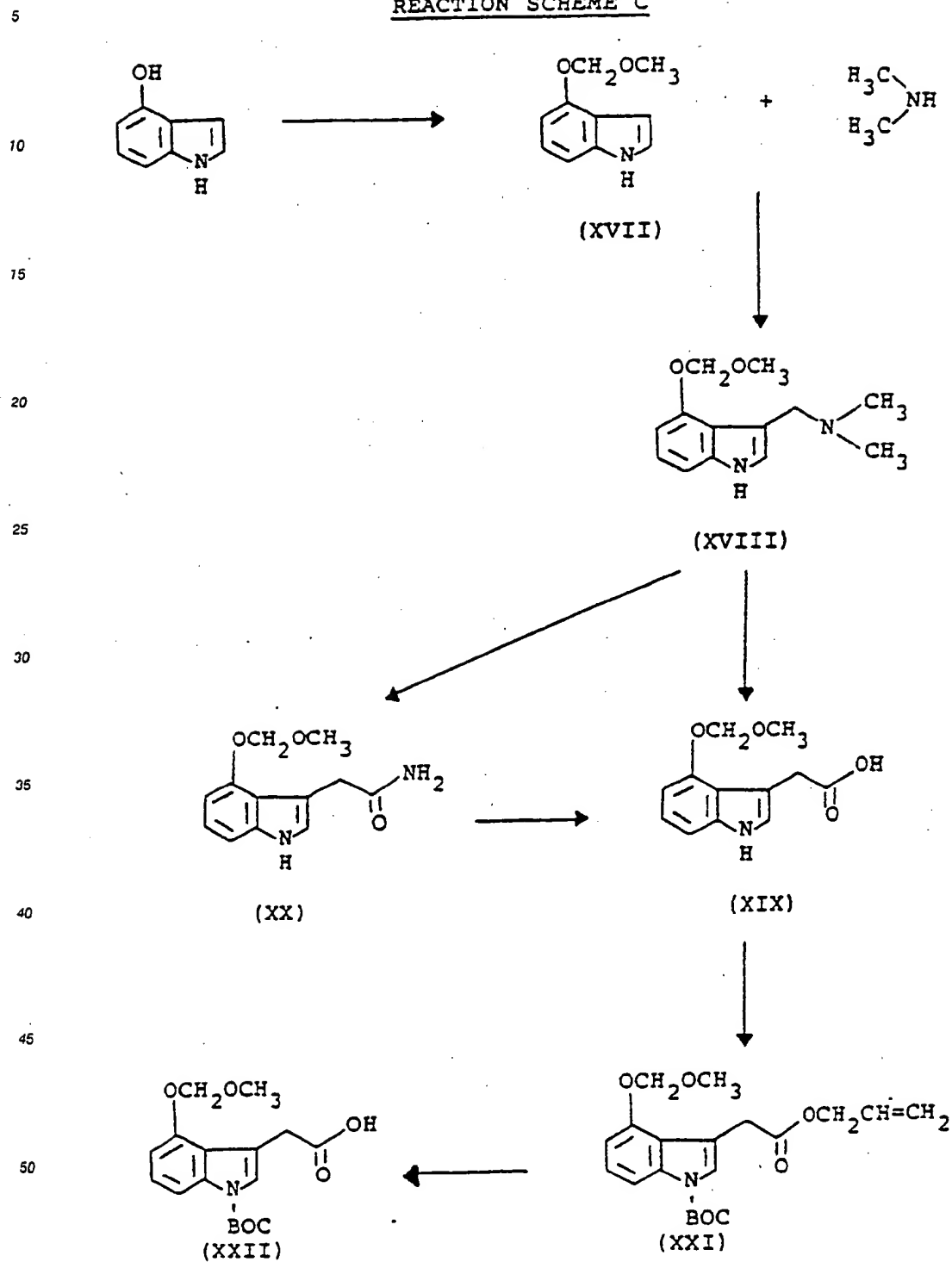




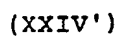
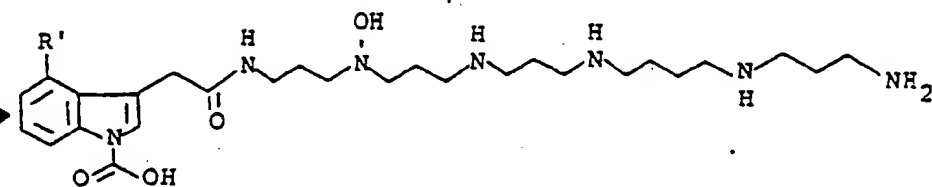
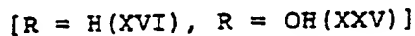
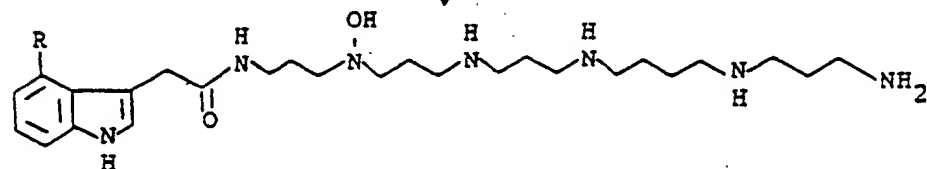
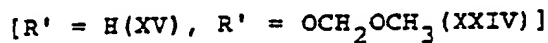
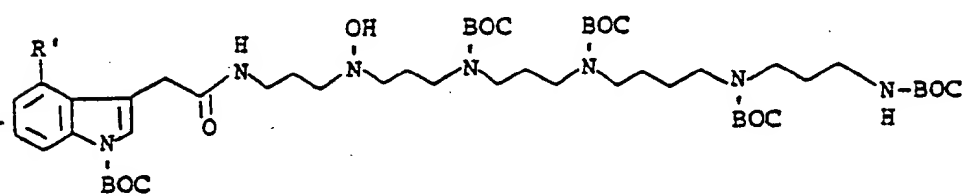
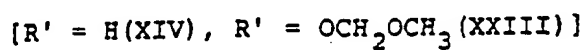
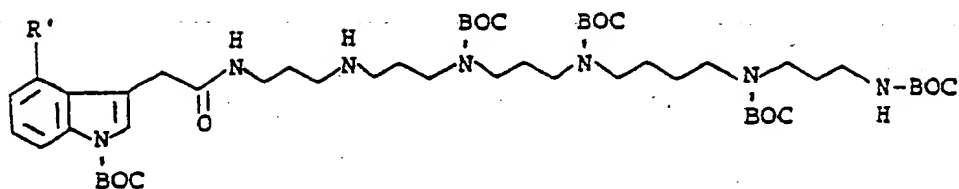
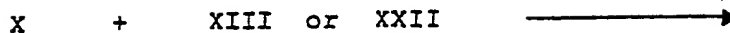
## REACTION SCHEME B

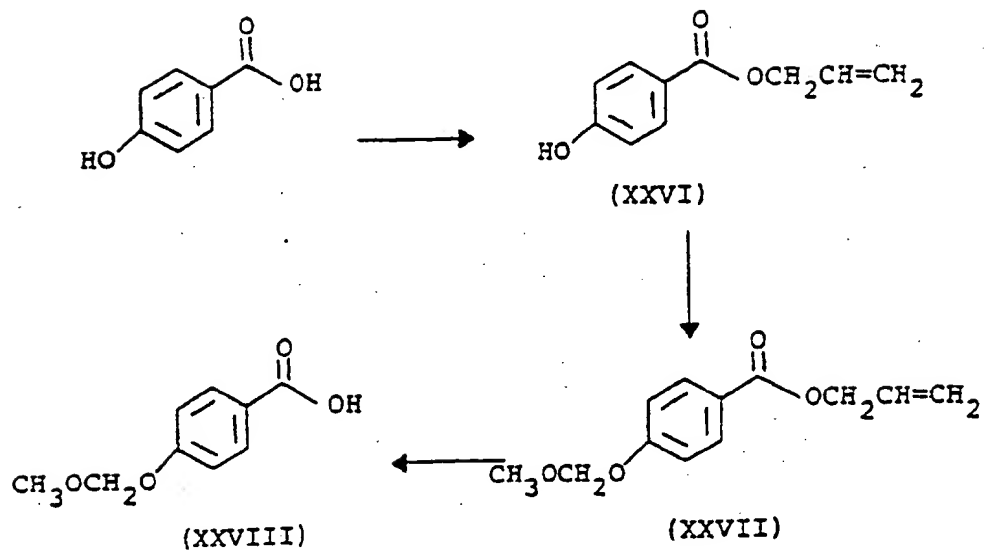
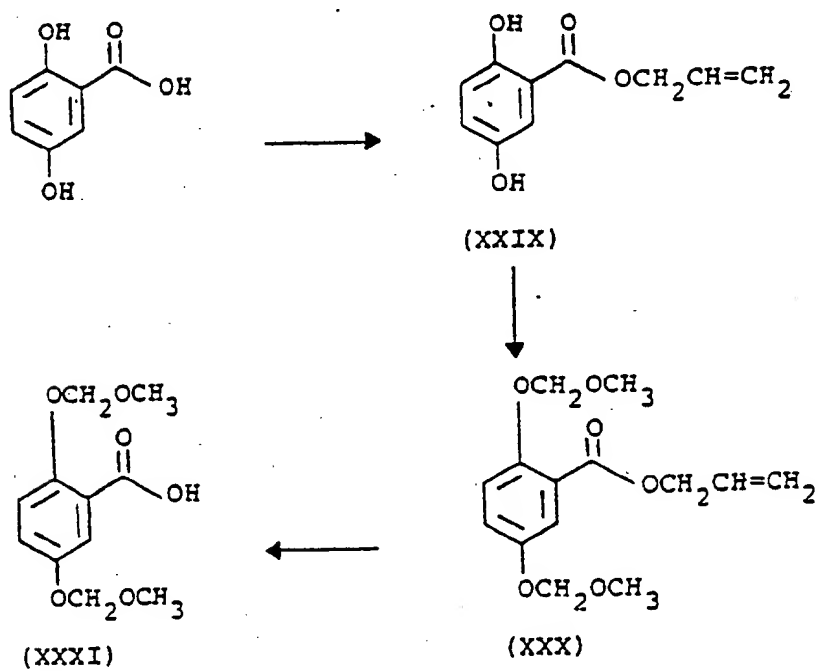


REACTION SCHEME C

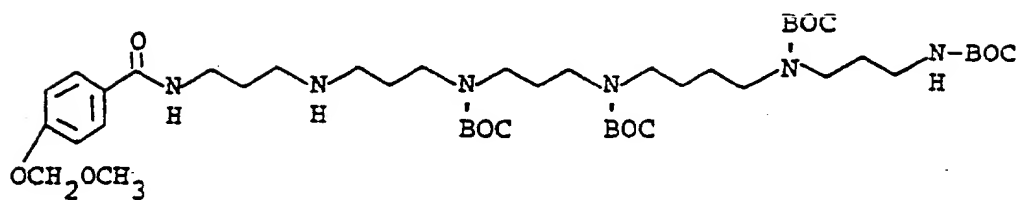
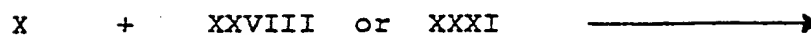


## REACTION SCHEME D



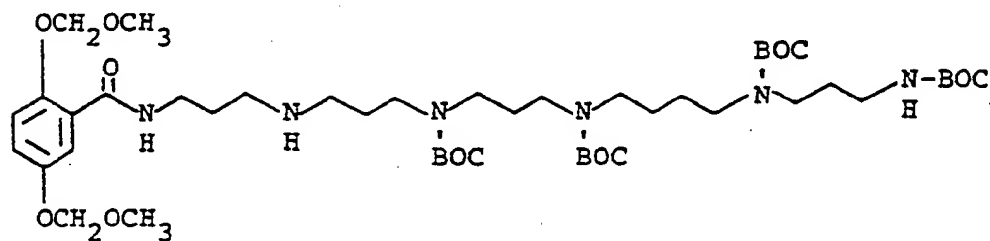
REACTION SCHEME EREACTION SCHEME F

## REACTION SCHEME G

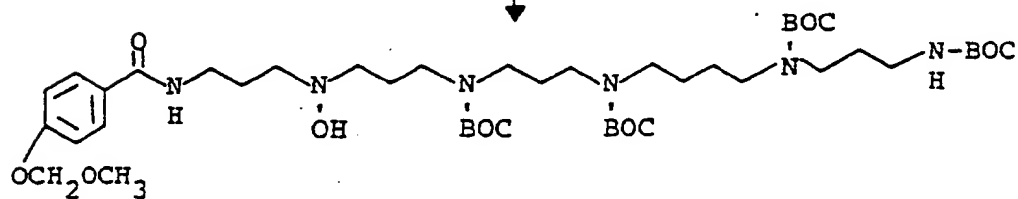


(XXXII)

or

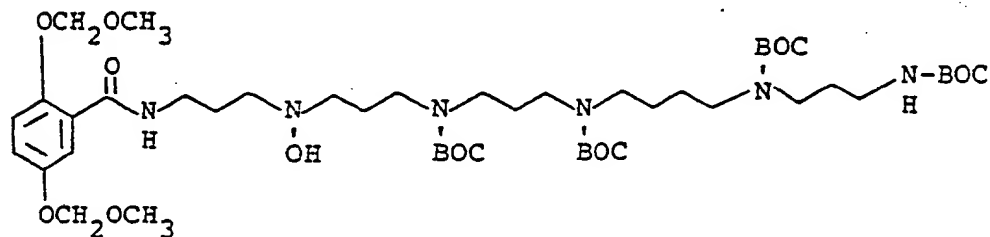


(XXXIII)



(XXXIV)

or



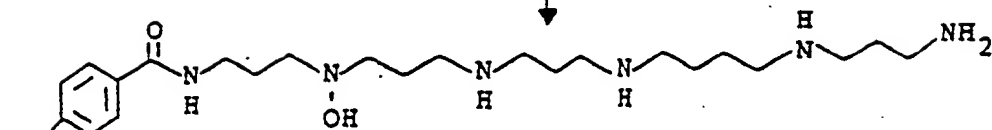
(XXXV)

REACTION SCHEME G (Continued)

5

(XXXIV) or (XXXV)

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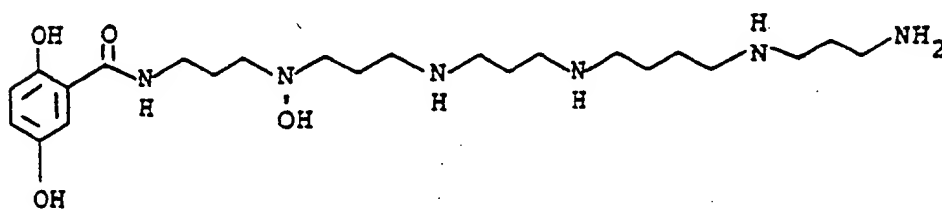
15

(XXXVI)

or

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(XXXVII)

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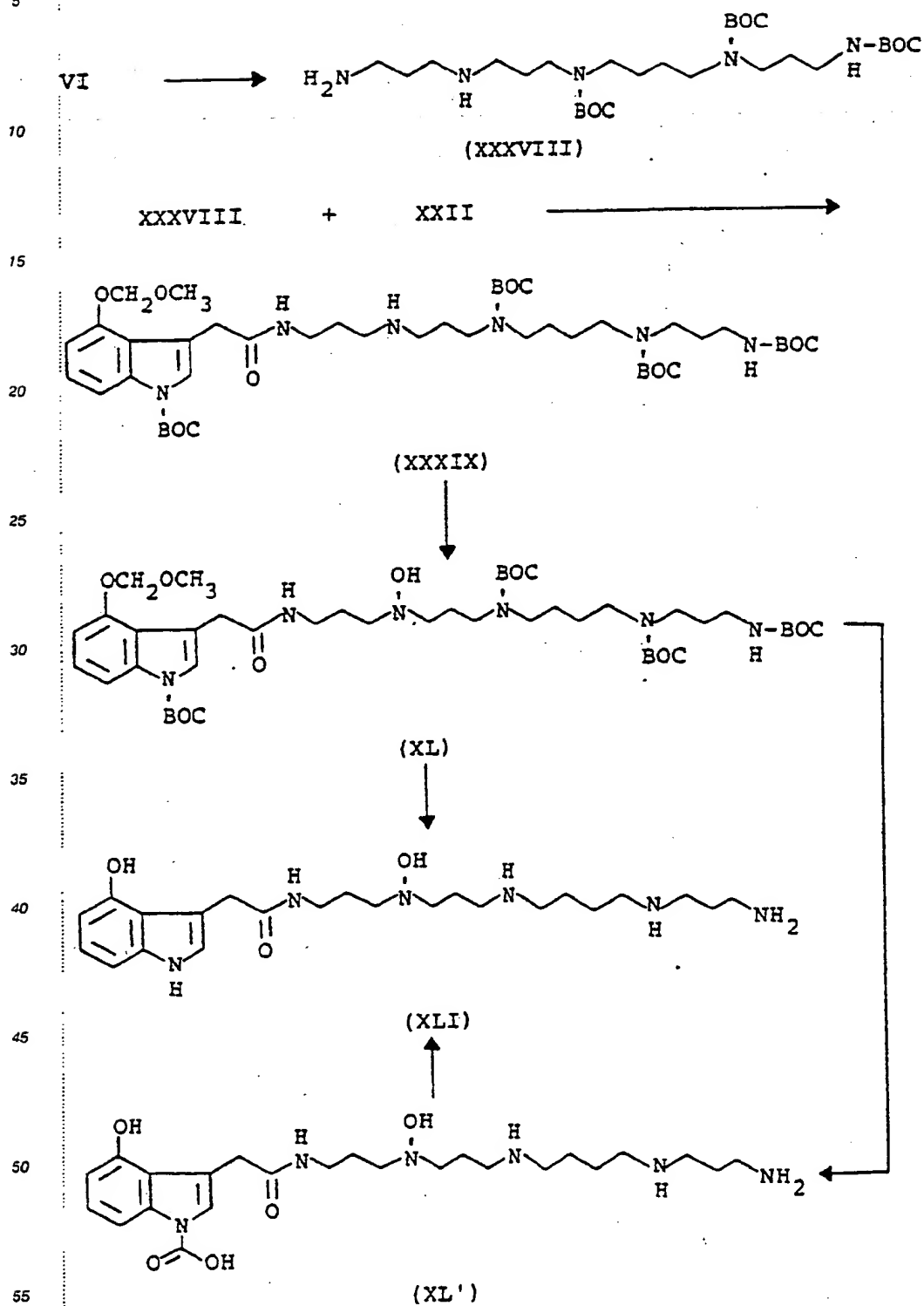
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REACTION SCHEME H





According to Reaction Scheme A, the polyamine intermediate compound of formula X is prepared through a sequence of steps beginning with diaminobutane. Reaction conditions suitable to prepare the intermediate compound of formula X according to Reaction Scheme A are given in Example 11, parts A to I. Reaction Scheme B illustrates a method for the preparation of the intermediate compound of formula XIII. Reaction conditions suitable to prepare that intermediate according to Reaction Scheme B are given in Example 11, parts J to L. Preparation of the intermediate compound of formula XXI is shown in Reaction Scheme C. Reaction conditions suitable for the preparation of the compound of formula XXI according to Reaction Scheme C are given in Example 12, parts A to F. Preparation of the polyamine compounds of this invention of the formulae XVI and XXV is shown in Reaction Scheme D. Reaction conditions suitable for the coupling of the intermediate compounds of formulae X and XIII or X and XXI and the subsequent preparation of compounds of the formulae XVI and XXV are given in Example 11, parts M to O and Example 12, parts G to I.

Reaction Scheme E and F illustrate methods for the preparation of the intermediate compounds XXVIII and XXXI, respectively. Reaction conditions suitable to prepare the intermediate according to Reaction Scheme E are given in Example 13, parts A to C. Reaction conditions suitable for the preparation of the intermediate according to Reaction Scheme F are given in Example 14, parts A to C. Preparation of the polyamine compounds of this invention of the formulae XXXVI and XXXVII is shown in Reaction Scheme G. Reaction conditions suitable for the coupling of the intermediate compounds of formula X and XXVIII or X and XXXI and the subsequent preparation of compounds of the formulae XXXVI and XXXVII are given in Example 13, parts D to F and Example 14, parts D to F, respectively.

Reaction Scheme H illustrates a method for the preparation of the polyamine compound of this invention of the formula XLI. Reaction conditions suitable for the preparation of the intermediate compound of formula XXXVIII, the coupling thereof to the intermediate compound of formula XXII and the subsequent preparation of the polyamine compound of this invention of the formula XLI are shown in Example 15.

The polyamines of this invention reversibly antagonize excitatory amino acid neurotransmitters, which neurotransmitters affect cells such as cells in the nervous system of a variety of organisms including invertebrates and vertebrates. The term vertebrates as used throughout is meant to include mammals. The term invertebrates as used throughout is meant to include, for example, insects, ectoparasites and endoparasites. The polyamine of fraction B<sub>1</sub>, as described above, also reversibly blocks calcium channels present in a variety of cells such as cells in the nervous and muscular, including cardiovascular, system of invertebrates and vertebrates.

The ability of the polyamines of the present invention to antagonize excitatory amino acid neurotransmitters is demonstrated by their ability to block N-methyl-D-aspartic acid-induced (NMDA) elevations of cGMP in neonatal rat cerebellums according to the following procedure. Cerebellums from ten 8-14 day old Wistar rats are quickly excised and placed in 4° C. Krebs/bicarbonate buffer, pH 7.4 and then chopped in 0.5 mm x 0.5 mm sections using a McIlwain tissue chopper (The Nickle Laboratory Engineering Co., Gomshall, Surrey, England). The resulting pieces of cerebellum are transferred to 100 ml of Krebs/bicarbonate buffer at 37° C. which is continuously equilibrated with 95:5 O<sub>2</sub>/CO<sub>2</sub>. The pieces of cerebellum are incubated in such a manner for ninety minutes with three changes of the buffer. The buffer then is decanted, the tissue centrifuged (1 min., 3200 r.p.m.) and the tissue resuspended in 20 ml of the Krebs/bicarbonate buffer. Then, 250 µl aliquots (approximately 2 mg) are removed and placed in 1.5 ml microfuge tubes. To those tubes are added 10 µl of the compound under study from a stock solution, followed by 10 µl of a 2.5 mM solution of NMDA to start the reaction. The final NMDA concentration is 100 µM. Controls do not have NMDA added. The tubes are incubated for one minute at 37° C. in a shaking water bath and then 750 µl of a 50 mM Tris-Cl, 5mM EDTA solution is added to stop the reaction. The tubes are placed immediately in a boiling water bath for five minutes. The contents of each tube then are sonicated for 15 seconds using a probe sonicator set at power level three. Ten microliters are removed and the protein determined by the method of Lowry, Anal. Biochem. 100:201-220 (1979). The tubes are then centrifuged (5 min., 10,000 xg), 100 µl of the supernatant is removed and the level of cyclic GMP (cGMP) is assayed using a New England Nuclear (Boston, Massachusetts) cGMP RIA assay according to the method of the supplier. The data is reported as pmole cGMP generated per mg. protein.

The polypeptides of this invention irreversibly block calcium channels present in a variety of cells such as cells in the nervous and muscular system of invertebrates and vertebrates.

The ability of the polyamine of Fraction B<sub>1</sub> and the polypeptides of Fractions G, H<sub>1</sub>, H<sub>2</sub>, I, J, K, L<sub>1</sub>, L<sub>2</sub> and M to block calcium channels is demonstrated by the following procedure. Dissociated rat neocortical neurons are suspended in Krebs bicarbonate buffer, pH 7.4 containing 2mM CaCl<sub>2</sub> and 1% bovine serum albumin (BSA). The neurons are sedimented by centrifugation and resuspended in the same buffer which

additionally contains 1  $\mu$ M fura2/AM (Sigma Chem. Co., P.O. Box 14508, St. Louis, Missouri 63178). The cells are incubated for 15 minutes at 37° C., washed and then incubated for another 15 minutes in the same buffer without fura2/AM. The cells are then washed in the above buffer now containing 1.5 mM CaCl<sub>2</sub> and equilibrated at room temperature as a concentrated cell suspension for about 5 to 10 minutes. To a quartz cuvette is added 1.2 ml of prewarmed (37° C.) BSA-free buffer containing 1.5 mM CaCl<sub>2</sub> and 10 mM glucose, then 0.3 ml of the concentrated cell suspension prepared above. The cuvette is placed in a thermostated (37° C.) holder equipped with a magnetic stirrer and the fluorescence is measured with a fluorescence spectrophotometer such as a Perkin Elmer 650-40 (Perkin Elmer, Wilton, Connecticut 06897). The fluorescence signal is allowed to stabilize for about one minute. Then 1-4  $\mu$ l of a stock solution of the compound under study in Krebs bicarbonate buffer at appropriate concentrations is added to the cuvette. Calibration of the fluorescent signals and fura-2 leakage correction are performed using the established procedures of Nemeth, et al., J. Biol. Chem. 262:5188 (1987). At the completion of each test, the maximum fluorescence value (F<sub>max</sub>) is determined by addition of ionomycin and the minimum fluorescence value (F<sub>min</sub>) is determined by the subsequent addition of 5 mM EGTA to chelate calcium. Employing the foregoing procedure, calcium channel blocking by a subject compound is shown to occur by a decrease in fluorescence upon addition of the subject compound.

Also within the scope of this invention are polypeptides having substantially the same amino acid sequence as the polypeptides of fractions G, H<sub>1</sub>, I, J, L<sub>1</sub>, L<sub>2</sub> and M and which have substantially the same calcium channel blocking activity as said polypeptides.

The polyamines of this invention are useful in antagonizing excitatory amino acid neurotransmitters, per se. As such, the polyamines are also useful in the control of invertebrate pests and in the treatment of excitatory amino acid neurotransmitter-mediated diseases and conditions in a mammal such as seizure, stroke, cerebral ischemia, neuronal degenerative disorders such as Alzheimer's disease and epilepsy. Said polyamines also are useful as psychotherapeutics in a mammal. Further, the polyamines are useful in the study of the physiology of cells including, but not limited to, cells of the nervous system.

The polyamine of Fraction B<sub>1</sub> and the polypeptides of this invention are useful as calcium channel blockers in cells, per se. As such, these compounds are also useful in the control of invertebrate pests and in the treatment of diseases and conditions mediated by calcium channels function in cells in a mammal such as angina, hypertension, cardiomyopathies, supraventricular arrhythmias, esophageal achalasia, premature labor and Raynaud's disease. Further, these compounds are useful in the study of the physiology of cells including, but not limited to, cells of the nervous and muscular system.

Also within the scope of this invention are the pharmaceutically-acceptable salts of the polyamines and polypeptides of this invention. Such salts are formed by methods well known to those skilled in the art. For example, acid addition salts of the polyamines and base salts of the polypeptides can be prepared according to conventional methods.

When a polyamine or polypeptide of this invention is to be administered to a mammal, it can be administered alone or in combination with pharmaceutically-acceptable carriers or diluents in a pharmaceutical composition according to standard pharmaceutical practice. The polyamines and polypeptides can be administered orally or parenterally with the parenteral route of administration being preferred for the polypeptides. Parenteral administration includes intravenous, intramuscular, intraperitoneal, subcutaneous and topical administration.

For oral use of a polyamine or polypeptide of this invention, the compound can be administered, for example, in the form of tablets or capsules, or as an aqueous solution or suspension. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch, and lubricating agents, such as magnesium stearate, are commonly added. For oral administration in capsule form, useful diluents are lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring agents can be added.

For intramuscular, intraperitoneal, subcutaneous and intravenous use, sterile solutions of the active ingredient are usually prepared, and the pH of the solutions should be suitably adjusted and buffered. For intravenous use, the total concentration of solutes should be controlled to render the preparation isotonic.

When a polyamine or polypeptide or salt thereof of this invention is used in a human subject, the daily dosage will normally be determined by the prescribing physician. Moreover, the dosage will vary according to the age, weight and response of the individual patient, as well as the severity of the patient's symptoms and the potency of the particular compound being administered.

When a polyamine or polypeptide or salt thereof of this invention is used in control of invertebrate pests, said compound is administered to said invertebrate directly or provided to the environment of said invertebrate. For example, a compound of this invention can be sprayed as a solution onto said invertebrate.

The amount of compound necessary for control of said invertebrate will vary according to the invertebrate and environmental conditions and will be determined by the person applying the compound.

When a polyamine or polypeptide or salt thereof of this invention is used in the physiological study of cells, said compound is administered to the cells according to methods well known to those skilled in the art. For example, said compound can be administered to cells in an appropriate physiological buffer. An appropriate concentration of the compounds of this invention for use in such studies is 100  $\mu$ M. However, the concentration of said compounds in such studies may be greater than or much less than 100  $\mu$ M. The amount of the compound administered will be determined by the person skilled in the art according to well known methods.

### EXAMPLE 1

#### Initial fractionation of whole venom of Agelenopsis aperta

Whole venom of Agelenopsis aperta, obtained from Natural Product Sciences Inc., Salt Lake City, Utah 84108 and which had been stored in the frozen state at about  $-78^{\circ}$  C., was thawed and 10 to 60  $\mu$ l amounts thereof, diluted to 200  $\mu$ l, and loaded onto a C-18 Vydac<sup>®</sup> (22 mm x 250 mm, 300 Å pore size, 10  $\mu$  particle size) column and eluted using a flow rate of 15 ml/min. and a solvent system using a linear gradient program of 5%  $\rightarrow$  20% B, 95%  $\rightarrow$  80% A [0  $\rightarrow$  30 min.] then 20%  $\rightarrow$  70% B, 80%  $\rightarrow$  30% A [30  $\rightarrow$  55 min.] where A is 0.1% aqueous TFA and B is acetonitrile. Peak detection was carried out monochromatically at 220-230 nm and fractions were collected with an ISCO/"FOXY" fraction collector and an ISCO 2159 peak detector. Fractions were collected from 20 minutes to 60 minutes. Based upon peak detection, the following fractions were collected:

Fraction	Elution Time
A	about 21 minutes
B	about 22.75 minutes
E	about 27.5 minutes
G	about 38 minutes
H	about 39 minutes
I	about 40 minutes
J	about 40 minutes
L	about 43 minutes
M	about 48.3 minutes

### EXAMPLE 2

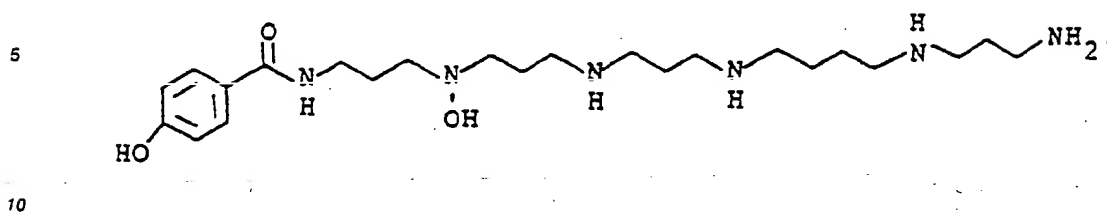
#### Fractionation of whole venom of Agelenopsis aperta to obtain Fraction A'

Whole venom of Agelenopsis aperta was obtained and loaded onto a C-18 Vydac<sup>®</sup> column as described in Example 1. The column was eluted using a flow rate of 15 ml/min. and a solvent system using a linear gradient program of 5%  $\rightarrow$  10% B, 95%  $\rightarrow$  90% A [0  $\rightarrow$  30 min.] where A is 0.1% aqueous TFA and B is acetonitrile. Peak detection and fraction collection were performed as described in Example 1. Fraction A' which eluted at about 12.5 minutes was obtained. Fraction A' was then prepared for spectral analysis by lyophilization from the eluent followed by lyophilization from distilled water, according to standard methods.

The structure of the compound which is comprised by fraction A' was then determined by the use of FAB MS. The data so obtained and the structure deduced therefrom are as follows:

A: FAB MS: M/Z 453 (M + 1)

Structure: Benzamide, N-(20-amino-4-hydroxy-4,8,12,17-tetraazaeicos-1-yl)-4-hydroxy

EXAMPLE 3Subfractionation of fraction A and determination of structures therein

20 Fraction A, obtained as described in Example 1, was loaded onto a C-4 Vydac® (10 mm x 250 mm, 300 Å pore size, 5 μ particle size) column and eluted therefrom using a flow rate of 4.0 ml/min. and a solvent system using a linear gradient program of 0% → 10% B, 100% → 90% A [0 → 30 min.] where A is 0.1% aqueous TFA and B is acetonitrile. Peak detection was accomplished using a Waters 990 diode array

25 detector and fraction collection was accomplished as described in Example 1. Two fractions were obtained as follows:

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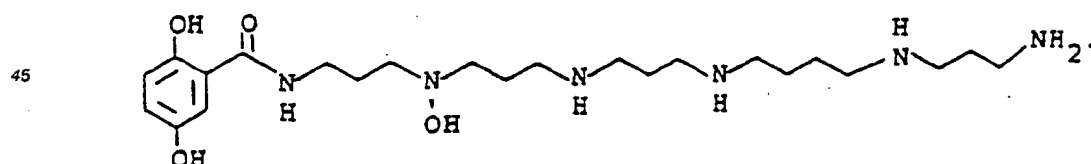
Fraction	Elution Time
A <sub>1</sub>	about 7 minutes
A <sub>2</sub>	about 9 minutes

35 Fractions A<sub>1</sub> and A<sub>2</sub> were then prepared for spectral analysis by lyophilization from the eluent followed by lyophilization from water, according to standard methods.

The structure of the compounds which are comprised by fractions A<sub>1</sub> and A<sub>2</sub> were then determined by the use of FAB MS. The data so obtained and structures deduced therefrom are as follows:

A<sub>1</sub>:

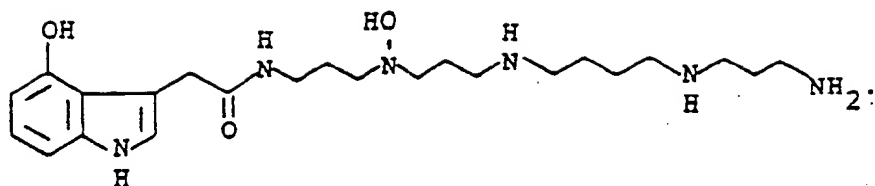
40 FAB MS: M/Z 469 (M + 1), high resolution: 469.3863 calc. for C<sub>23</sub>H<sub>45</sub>N<sub>6</sub>O with 2.0 mmu error  
 structure: benzamide, N-(20-amino-4-hydroxy-4,8,12,17-tetraazaeicos-1-yl)-2,5-dihydroxy

A<sub>2</sub>:

FAB MS: M/Z 449 (M + 1), 433, 376, 260, 203

Structure: 1H-indole-3-acetamide, N-(16-amino-4-hydroxy-4,8,13-triazahehexadec-1-yl)-4-hydroxy

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#### EXAMPLE 4

##### Subfractionation of fraction B and determination of structures therein

Fraction B, obtained as described in Example 1, was loaded onto a C-4 Vydac® (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) column and eluted off using a flow rate of 15 ml/min. and a solvent system using a non-linear gradient program of 0% → 0% B, 100% → 100% A [0 → 5 min.], then 0% → 10% B, 100% → 90% A [5 → 20 min.] (Waters curve 1) then 10% → 20% B, 90% → 80% A [20 → 30 min.] (Waters curve 6) then 20% → 50% B, 80% → 50% A [30 → 40 min.] (Waters curve 11) where A is 0.1% aqueous TFA and B is acetonitrile. Peak detection was accomplished using a Waters 990 diode array detector and fraction collection was accomplished as described in Example 1. Two fractions were obtained as follows:

Fraction	Elution Time
B <sub>1</sub>	about 18.5 minutes
B <sub>2</sub>	about 21.5 minutes

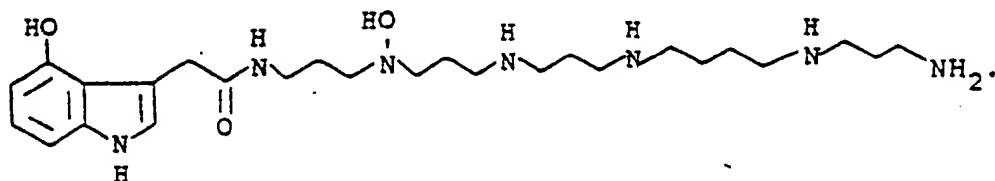
The fractions were then prepared for spectral analysis by lyophilization from the eluent followed by lyophilization from water according to standard methods.

The structure of the compound which is comprised by fraction B<sub>1</sub> was determined by the use of FAB MS and the results thereof are as follows:

FAB MS: M/Z 506 (M+1), 489, 461, 433, 378, 333, 260, 231, 215, 203, 155, 119

high resolution: 506.3810 calc. for C<sub>26</sub>H<sub>48</sub>N<sub>7</sub>O<sub>3</sub>

Structure: 1H-indole-3-acetamide, N-(20-amino-4-hydroxy-4,8,12,17-tetraazaeicos-1-yl)-4-hydroxy



The molecular weight of the compound which is comprised by fraction B<sub>2</sub> was determined by the use of FAB MS and the results thereof are as follows:

FAB MS:

high resolution: 505.3861 calc. for C<sub>27</sub>H<sub>48</sub>N<sub>6</sub>O<sub>3</sub>.

#### EXAMPLE 5

Structure of compound comprised by fraction E

Fraction E, obtained as described in Example 1, was prepared for spectral analysis by lyophilization from the eluent followed by lyophilization from water according to standard methods. The structure of the compound comprised by fraction E was determined using FAB MS, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR. The data so obtained and the structure deduced therefrom are as follows:

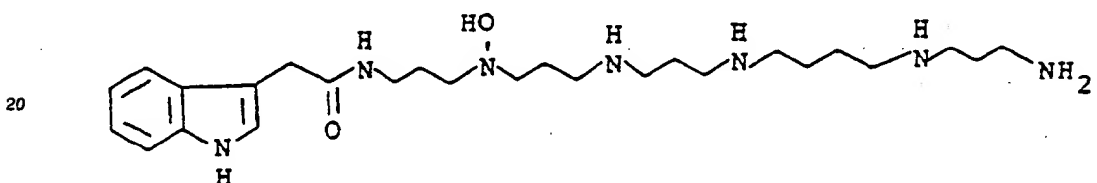
FAB MS:M/Z 490 (M + 1), 472, 433, 362, 333, 260, 215, 203, 177, 155, 119

high resolution: 490.3860 calc. for C<sub>26</sub>H<sub>48</sub>N<sub>7</sub>O<sub>2</sub> with 0.6 mmu error

<sup>1</sup>H-NMR (500 Mhz, d6-DMSO): 10.89 (s, 1H), 8.90-7.85 (m, 6H), 7.55 (d, J = 8.0 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.19 (s, 1H), 7.08 (dd, J = 8.0 Hz, J = 8.0 Hz, 1H), 6.98 (dd, J = 8.0 Hz, J = 8.0 Hz, 1H), 3.50 (s, 2H), 3.13 (m, 2H), 3.03-2.82 (m, 18H), 1.88 (m, 6H), 1.78 (m, 2H), 1.62 (m, 4H)

<sup>13</sup>C-NMR (125.76 Mhz, d6-DMSO): 170.94, 136.10, 127.17, 123.75, 120.92, 118.67, 118.26, 111.33, 108.97, 57.39, 57.07, 46.12, 46.12, 44.06, 43.89, 43.89, 36.52, 36.22, 32.78, 26.71, 23.79, 23.06, 22.65, 22.65, 22.45

Structure: 1H-indole-3-acetamide, N-(20-amino-4-hydroxy-4,8,12,17-tetraazecos-1-yl)



Alternatively and preferably, whole venom was fractionated as described in Example 1 except that the solvent system and linear gradient program used were 0% → 20% B, 100% → 80% A [0 → 30 min.] with peak detection at 220 nm. The fraction which eluted at about 26.0 minutes was loaded onto a Dynamax Phenyl column (4.6 mm x 250 mm, 60 Å pore size, 8 μ particle size) and eluted using a flow rate of 1 ml/min. and isocratic conditions of 10% B, 90% A where A and B are as described in Example 1. Peak detection was accomplished using a Waters 990 diode array detector (λ = 220 nm) and fractions were collected as described in Example 1. The fraction which eluted at about 55.27 minutes was lyophilized from the eluent followed by lyophilization from water according to standard methods to yield the compound of this Example.

EXAMPLE 6Subfractionation of fraction G

Fraction G, obtained as described in Example 1 was loaded onto a C-18 Vydac® (22 mm x 250 mm, 300 Å pore size, 10 μ particle size) column and eluted off using a flow rate of 10 ml/min. and a solvent system using a non-linear gradient program of 20% → 30% B, 80% → 70% A [0 → 40 min.] (Waters curve 6) and employing a Waters 990 diode array detector with fractions being collected as described in Example 1. Fraction G, after subfractionation as described above, eluted from the column at about 22 minutes. That fraction, which comprises a polypeptide, then was prepared for sequencing by lyophilization from the eluent followed by lyophilization from water, according to well known procedures.

Amino acid analysis of the alkylated polypeptide of the fraction was obtained using the Waters Pico-Tag method according to manufacturer's specifications. Sequence data was collected from an Applied Biosystems model 470A Protein/Peptide sequencer (Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, California 94404) with aqueous TFA conversion. Analysis of the resulting phenylthiohydantoin amino acids was accomplished on line with an Applied Biosystems model 120A PTH analyzer or off line on a Dupont Zorbax PTH column (Biomedical Product Department, Chromatography Products, E. I. DuPont de Nemours and Co., Inc., 1007 Market Street, Wilmington, Delaware 19898).

As a result of amino acid analysis and FAB MS of the entire polypeptide it was determined that the amino-terminal amino acid sequence of part of the polypeptide comprised by fraction G was determined as:

H<sub>2</sub>N-glu-lys-gly-leu-pro-glu-gly-ala-glu-cys-asp-gly-asn-glu-ser-asp-cys-lys-cys-ala-gly-gln-trp-ile-lys-cys-arg-cys-pro-trp-lys-trp-his-ile-thr-gly-glu-gly-pro-cys-thr-cys-glu-arg-gly-leu-lys-lys-thr-cys-ile-ser-lys-leu-ser-asp-pro-asn-arg-asn-glu-trp-leu-ser-; and FAB MS: 7267.

### EXAMPLE 7

#### Subfractionation of fraction H and determination of structures therein

Fraction H, obtained as described in Example 1, was loaded onto a C-18 Vydac® (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) column and eluted off using a flow rate of 10 ml/min. and a solvent system using a non-linear gradient program of 20% → 25% B, 80% → 75% A [0 → 30 min.] (Waters curve 6) then 25% → 50% B, 75% → 50% A [30 → 45 min.] (Waters curve 11), where A is 0.1% TFA and B is acetonitrile, and employing a Waters 990 diode array detector with fractions being collected as described in Example 1. Of the fractions collected, the fractions of interest were:

Fraction	Elution Time
H <sub>2</sub>	about 36 minutes
H <sub>3</sub>	about 41 minutes

Fractions H<sub>2</sub> and H<sub>3</sub>, which comprise polypeptides, then were prepared for sequencing and sequenced according to the procedures described in Example 6.

The amino terminal amino acid sequence of part of the polypeptide comprised by fraction H<sub>2</sub> was determined as:  
H<sub>2</sub>N-ala-lys-ala-leu-pro-pro-gly-ser-val-cys-asp-gly-asn-glu-ser-asp-cys-lys-cys-tyr-gly-lys-trp-his-lys-cys-arg-cys-pro-trp-lys-trp-his-phe-thr-gly-glu-gly-pro-cys-thr-cys-glu-lys-gly-met-lys-his-thr-cys-ile-thr-lys-leu-his-cys-pro-asn-lys-ala-glu-trp-gly-leu-asp-trp-; and the molecular weight of the entire polypeptide was determined by Ion-Spray MS to equal 7793.

### EXAMPLE 8

#### Subfractionation of fractions I and J and determination of structures therein

Fractions I and J, obtained as described in Example 1 and which were not adequately separated due to the similar elution times as shown in Example 1, were loaded together onto a C-4 Vydac® (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) column and eluted off using a flow rate of 10 ml/min. and a solvent system using a non-linear gradient program of 20% → 30% B, 80% → 70% A [0 → 30 min.] (Waters curve 6), then 30% → 50% B, 70% → 50% A [30 → 45 min.] (Waters curve 11) where A is 0.1% aqueous TFA and B is acetonitrile. Peak detection and fraction collection were accomplished according to the procedures described in Example 6. Of the fractions collected, the fractions of interest were:

Fraction	Elution Time
I	about 22 minutes
J	about 27.5 minutes

Fractions I and J, which comprise polypeptides, were prepared for sequencing and sequenced

according to the procedures described in Example 6.

The amino acid sequence and FAB MS of the polypeptide comprised by fraction I were determined as:  
 $\text{H}_2\text{N}$ -asp-cys-val-gly-glu-ser-gln-gln-cys-ala-asp-trp-ala-gly-pro-his-cys-cys-asp-gly-tyr-tyr-cys-thr-cys-arg-tyr-phe-pro-lys-cys-ile-cys-val-asn-asn-asn-CONH<sub>2</sub> and FAB MS: 4158.

The amino-terminal amino acid sequence of part of the polypeptide and FAB MS of the entire polypeptide comprised by fraction J was determined as:

$\text{H}_2\text{N}$ -asp-glu-pro-cys-ile-pro-leu-gly-lys-ser-cys-ser-trp-lys-ile-gly-thr-pro-tyr-cys-cys-pro-his-pro-asp-asp-ala-gly-arg-arg-thr-trp-cys-leu-val-asp-tyr-ser-arg-phe-val-thr-ile-cys-ser-gly-arg-lys-tyr-CONH<sub>2</sub>; and FAB MS: 5506.

#### EXAMPLE 9

##### Subfractionation of fraction L and determination of structures therein

Fraction L, obtained as described in Example 1, was loaded onto a C-18 Vydac® (10 mm x 250 mm, 300 Å pore size, 5 µ particle size) column and eluted off using a flow rate of 3.5 ml/min. and a solvent system using a linear gradient program of 25% → 40% B, 75% → 60% A [0 → 30 min.] where A is 0.1% aqueous TFA and B is acetonitrile. Peak detection and fraction collection were accomplished according to the procedures described in Example 6. Of the fractions collected, the fractions of interest were:

Fraction	Elution Time
L <sub>1</sub>	about 20.25 minutes
L <sub>2</sub>	about 22.5 minutes

Fractions L<sub>1</sub> and L<sub>2</sub>, which comprise polypeptides, were prepared for sequencing and sequenced according to the procedures described in Example 6.

The amino-terminal amino acid sequence of part of the polypeptide comprised by fraction L<sub>1</sub> was determined as:

$\text{H}_2\text{N}$ -ile-val-gly-gly-lys-thr-ala-lys-phe-gly-asp-tyr-pro-trp-met-val-ser-ile-gln-gln-lys-asn-lys-lys-gly-gly-phe-asp-and the molecular weight of the entire polypeptide was determined by gel electrophoresis according to known methods to be about 20,000.

#### EXAMPLE 10

##### Determination of structure of compound comprising fraction M

Fraction M, obtained as described in Example 1 and which comprises a polypeptide, was prepared for sequencing and sequenced according to the procedures described in Example 6.

The amino-terminal amino acid sequence of part of the polypeptide comprised by fraction M was determined as:

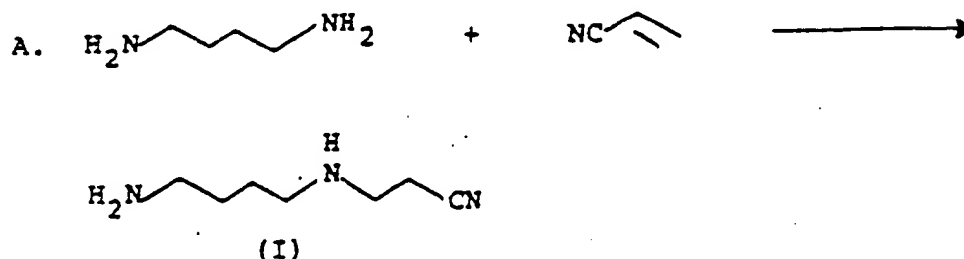
$\text{H}_2\text{N}$ -glu-ala-thr-glu-ala-ala-lys-val-leu-ser-asn-leu-asp-glu-thr-val-asp-pro-and the molecular weight of the entire polypeptide was determined by gel electrophoresis according to known methods to be about 80,000.

#### EXAMPLE 11

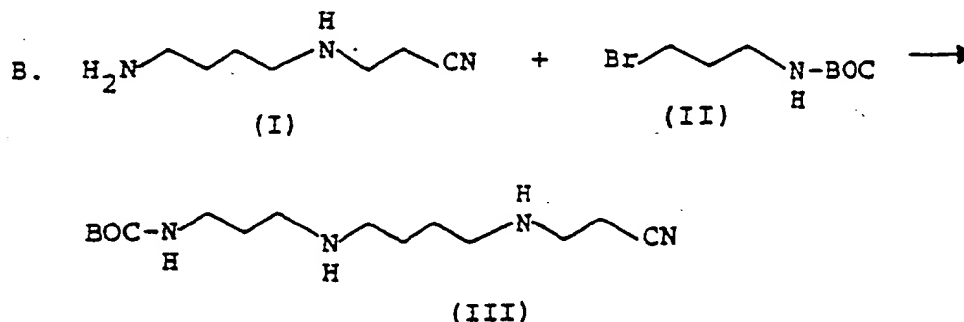


1H-Indole-3-acetamide, N-(20-amino-4-hydroxy-4,8,12,17-tetraazecos-1-yl

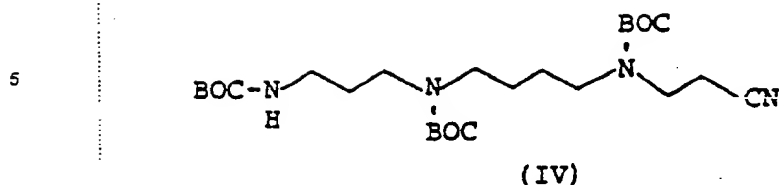
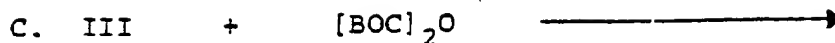
Synthesis of the title compound, ascertained to be comprised by fraction E as described in Example 4,  
 5 was accomplished as described below.



Under nitrogen atmosphere, 13.22 g (0.15 mole) diaminobutane and 3.5 ml methanol were stirred and  
 9.94 ml (0.15 mole) of acrylonitrile was added via a syringe pump over a two hour period with cooling to 0-  
 5° C. The mixture, after about 18 hours, was chromatographed on 600 g silica gel using a solvent system  
 20 of 3:1  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (2 liters) followed by 3:1  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  containing 5% by volume isopropylamine (2  
 liters). The fractions containing the coupled product were concentrated by evaporation in vacuo and yielded  
 a yellow viscous oil. NMR analysis verified the product to be the coupled product of formula (I) shown  
 above.



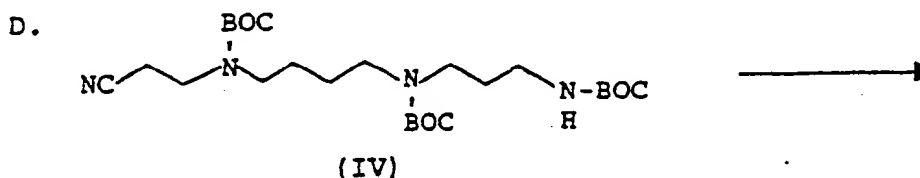
Under nitrogen atmosphere, 5.78 g (0.041 mole) of compound of formula I, prepared as described in  
 40 part A above, was added to 75 ml of  $\text{CH}_3\text{CN}$  and 11.48 g of KF Celite and stirred. To the stirring mixture  
 was added a solution of 9.75 g (0.041 mole) of compound of formula II, prepared according to the  
 procedure described in Preparation A, in 25 ml of  $\text{CH}_3\text{CN}$ . The reaction was heated to reflux and monitored  
 by TLC (3:1  $\text{CH}_2\text{Cl}_2/\text{MeOH}$ ). After three hours, the reaction was allowed to cool and stand at room  
 45 temperature. The Celite was then filtered off and the filter-cake was washed well with dichloromethane. The  
 filtrate was concentrated in vacuo. The crude product contained in the concentrated filtrate was chromatog-  
 raphed on silica gel using 2 liters of 3:1  $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , then 2 liters of 3:1  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  and 10 ml  
 isopropylamine, then 2 liters of 3:1  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  and 30 ml isopropylamine. The product eluted from the  
 column after the column became saturated with isopropylamine but was still impure. All product fractions  
 50 were combined and concentrated. Silica gel was prepared by slurring 500 g of silica gel in  $\text{CH}_2\text{Cl}_2$  and  
 125 ml isopropylamine and then used to pack a column in the standard manner. The crude product was  
 loaded onto the column using dichloromethane. Then, 500 ml of dichloromethane was run through the  
 column, followed by one liter of 3:1  $\text{CH}_2\text{Cl}_2/\text{MeOH}$ . The product fractions were combined and concentrated  
 in vacuo to yield 1.5 g of product of formula III, above. Another 2 g of product of formula III was eluted off  
 55 the column using one liter of 3:1  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  and 30 ml isopropylamine.



10

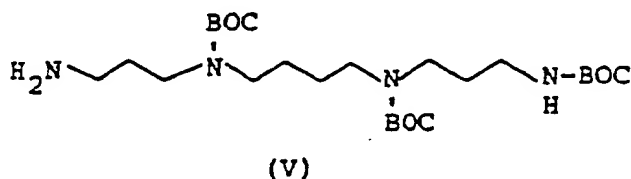
Under nitrogen atmosphere, 3.5 g (11.8 mmoles) of compound of formula III, prepared as described in part B, above, and 150 ml dichloromethane were stirred and 5.2 g (23.6 mmoles) of di-*t*-butyldicarbonate were added. The mixture was stirred for approximately 68 hours at room temperature. The mixture was then concentrated in *vacuo* and chromatographed using 400 g of silica gel and a 60:40 hexane/ethyl acetate solvent to yield 5.62 g of compound of formula IV as an oil.

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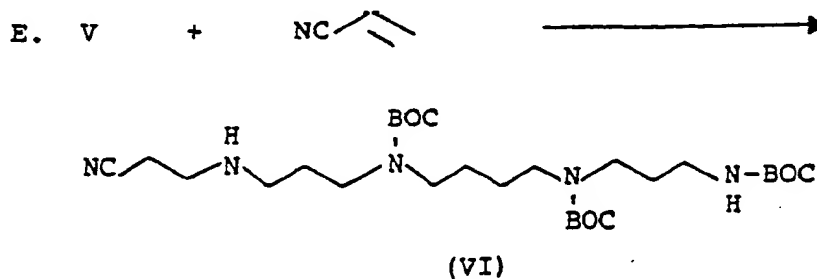


30

Under nitrogen atmosphere, 3.0 g of compound of formula IV, prepared as described in part C, above, was dissolved in 30 ml of acetic acid in a 250 ml Parr® bottle. To that solution was added 3.0 g of Pd(OH)<sub>2</sub>/carbon and the mixture was hydrogenated at p.s.i. H<sub>2</sub> pressure for 2 hours. The catalyst was removed by filtration and the cake was washed well with acetic acid. The filtrate was concentrated, taken up in 75 ml of dichloromethane, washed twice with 75 ml of 1N NaOH and dried over K<sub>2</sub>CO<sub>3</sub>. The solution was concentrated in *vacuo* to yield 2.86 g of product of formula V above.

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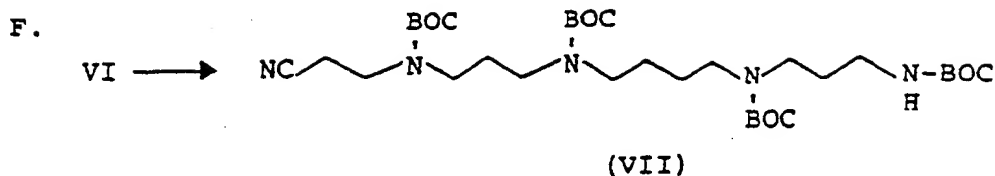


45

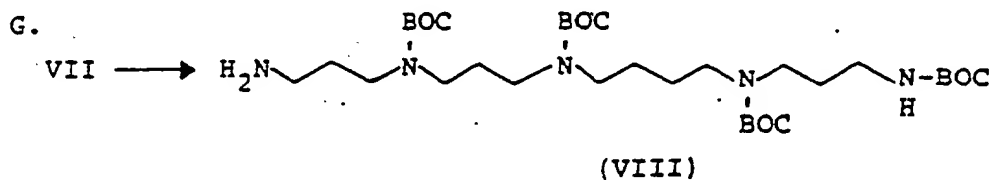
50

Under nitrogen atmosphere, 2.86 g (5.7 mmoles) of compound of formula V, prepared as described in part D, above, was dissolved in 75 ml methanol. With stirring, 0.41 ml (6.3 mmoles) of acrylonitrile was added to the mixture and the reaction was stirred overnight. The reaction mixture was then concentrated, reconcentrated three times from 30 ml dichloromethane and stripped of solvent in *vacuo* to yield 3.18 g of compound of formula VI as an oil.

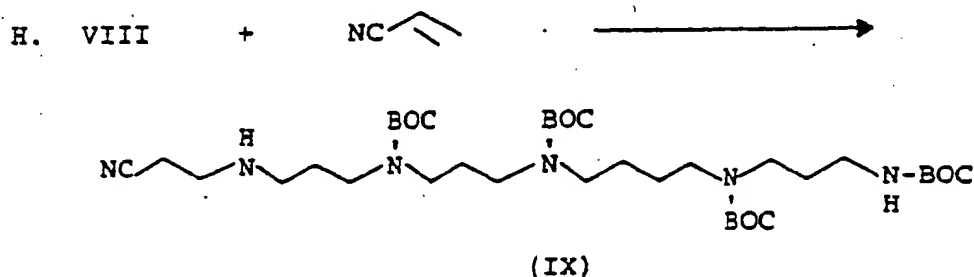
55



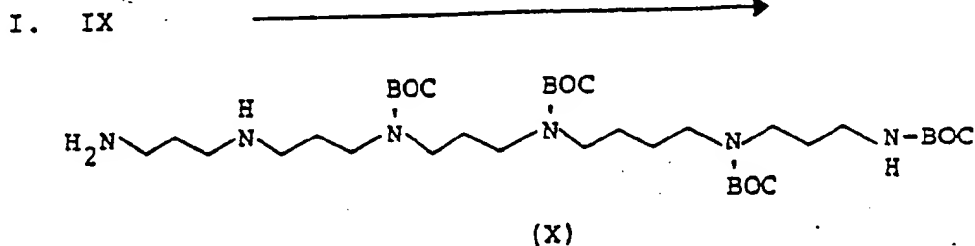
Under nitrogen atmosphere, 3.18 g (5.7 mmoles) of compound of formula VI, prepared as described in part E, above, was combined with 100 ml of dichloromethane and stirred into solution. To that solution was added 1.37 g (6.3 mmoles) of di-*t*-butyldicarbonate and the reaction was stirred for 90 minutes at room temperature. The reaction mixture was then concentrated and chromatographed on 300 g silica gel using 60:40 hexane/ethyl acetate as the eluent. The product fractions were combined, concentrated, extracted with 3 x 20 ml dichloromethane and stripped of solvent in vacuo to yield 3.12 g of product of formula VII, above.



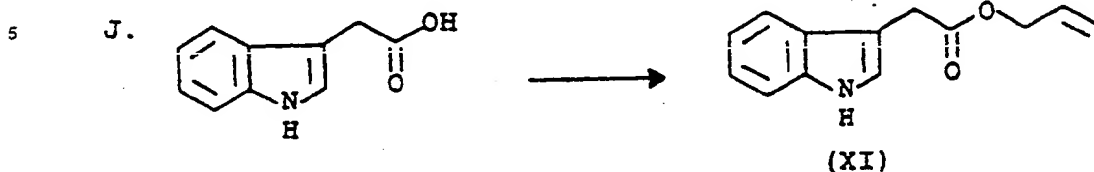
Following the procedure described in part D, above, 3.12 g (4.76 mmoles) of compound of formula VII, prepared as described in part F, above, was dissolved in 30 ml acetic acid and hydrogenated at 55 p.s.i. for 2 hours in the presence of 3.0 g Pd(OH)<sub>2</sub>/carbon to yield 3.02 g of product of formula VIII, above, as an oil.



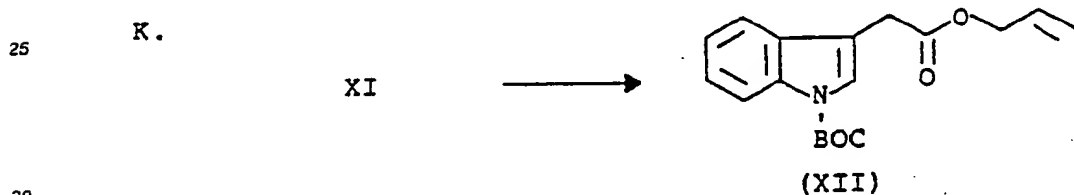
Under nitrogen atmosphere, 1.0 g (1.5 mmoles) of compound of formula VIII, prepared as described in part G, was dissolved in 10 ml methanol. To that solution was added 0.1 ml (1.67 mmoles) of acrylonitrile and the reaction was stirred overnight. Then, the reaction mixture was concentrated, reconstituted three times from 20 ml of dichloromethane and stripped of solvent in vacuo to yield 1.0 g of product of formula IX, above.



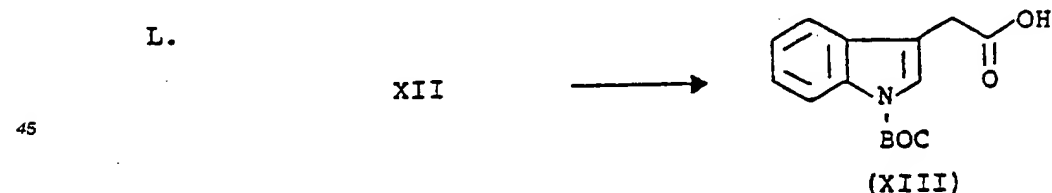
Following the procedure described in part D, above, 1.0 g (1.4 mmoles) of compound of formula IX, prepared as described in part H, above, was dissolved in 30 ml acetic acid and hydrogenated at 50 p.s.i. for 2.5 hours to yield 0.85 g of product of the formula X, above.



10 A solution containing 6.78 g (20 mmoles) tetrabutylammonium hydrogen sulfate and 75 ml H<sub>2</sub>O was prepared by stirring and 3.36 g (40 mmoles) of NaHCO<sub>3</sub> was added as foaming would allow. Then, 3.5 g (20 mmoles) of indole acetic acid was added, the mixture was stirred for 5 minutes and 75 ml of CHCl<sub>3</sub> was added. The mixture was stirred for another 5 minutes and the bottom two layers were separated. The aqueous layer was salted with Na<sub>2</sub>SO<sub>4</sub> and extracted with CHCl<sub>3</sub>. All CHCl<sub>3</sub> extracts were combined and dried over Na<sub>2</sub>SO<sub>4</sub>. The resulting clear amber colored solution was concentrated, chased with 3 x 75 ml acetone and finally dissolved in 75 ml acetone. Then, 1.9 ml (22 mmoles) of allyl bromide was added and the mixture was allowed to stand for 30 minutes. The mixture was then concentrated and chromatographed on silica gel using 3:1 hexane/ethyl acetate to yield 3.57 g of product of formula XI, above, as a light yellow oil.

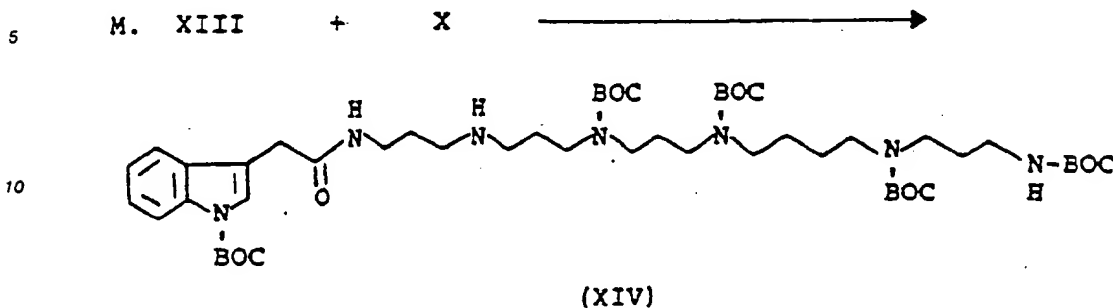


30 Following the procedure of Angew. Chem. Int. Ed. Engl. 23:298 (1984), 2.15 g (10 mmoles) of compound of formula XI, prepared as described in part J, above, was combined with 20 ml acetonitrile and 2.61 g (12 mmoles) of di-*t*-butyldicarbonate was added to the mixture. Then, 0.122 g (1 mmole) of 4-(*N,N*-dimethylamino)pyridine was added and the reaction permitted to proceed for 15 minutes. The mixture was then diluted out to 125 ml with ethyl acetate, washed 2 x dilute HCl, 3 x 25 ml H<sub>2</sub>O, 1 x brine, dried and concentrated. The product was purified by chromatography on silica gel using 14.5:1 hexane/ethyl acetate as the eluent to yield 2.87 g of compound of the formula XII, above, as a colorless oil.



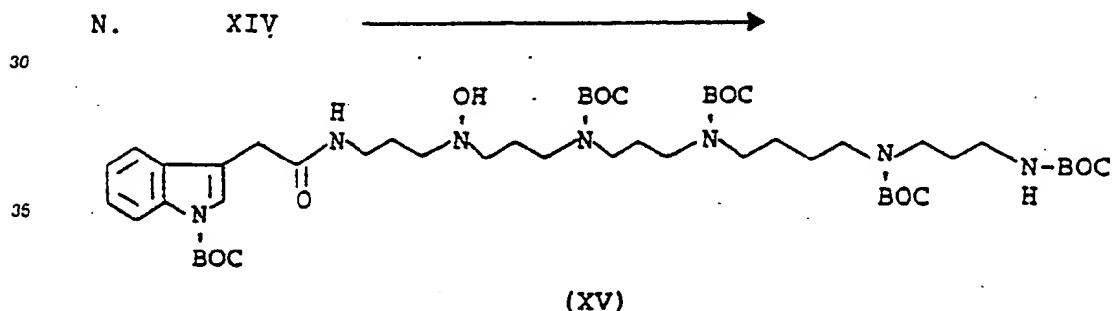
50 To 3.27 g (10.4 mmoles) of compound of formula XII, prepared according to part K, above, in 20 ml dichloromethane was added 7.4 ml of sodium 2-ethylhexanoate in ethyl acetate (0.231 g/ml). Then, 0.5 g  $\text{P}(\text{O})\text{C}_3\text{H}_7$  and 0.5 g  $(\text{P}(\text{O})\text{C}_3\text{H}_7)_2\text{Pd}$  were added and the mixture was stirred for 60 minutes. The mixture was then concentrated, taken up in 150 ml ethyl acetate and washed 5 x 25 ml H<sub>2</sub>O. The aqueous extracts were combined and back extracted with 1 x 25 ml ethyl acetate and 1 x 25 ml diethyl ether. To the aqueous layer was added 75 ml of fresh ethyl acetate and then acidified to pH 3. The ethyl acetate layer was separated and the aqueous layer was extracted with 1 x 50 ml fresh ethyl acetate. The two ethyl acetate extracts were combined and washed with 2 x 20 ml H<sub>2</sub>O, then 1 x 20 ml brine, dried, concentrated, chased

with 3 x 30 ml hexane where crystals formed during the second chase. The mixture was diluted out to 75 ml with hexane. The solids were filtered, washed well with hexane and air dried to yield 1.37 g of white solids. NMR confirmed the title structure of formula XIII.



15            Under nitrogen atmosphere, 200 mg (.73 mmoles) of the compound of formula XIII was combined with 25 ml dichloromethane, 84 mg (0.73 mmoles) N-hydroxysuccinimide and 150 mg (0.73 mmoles) dicyclohexylcarbodiimide whereupon a precipitate formed almost immediately. The mixture was stirred 3 hours. Then, the dicyclohexylurea was filtered off and to the filtrate was added dropwise a 40 ml solution of compound of the formula X, prepared as described in Example 11, Parts A-I in dichloromethane. The mixture was stirred for 12 hours then washed with 0.1 NaOH and dried over  $K_2CO_3$ , concentrated and chromatographed using 150 g silica gel and eluting with 9:1 dichloromethane/methanol (0.5 liters) to remove

25            impurities then with 9:1:0.1 dichloromethane/methanol/isopropylamine (2 liters). The product was then concentrated, chased with dichloromethane and further concentrated to yield 400 mg of the compound of formula XIV, confirmed by 300 MHz NMR.

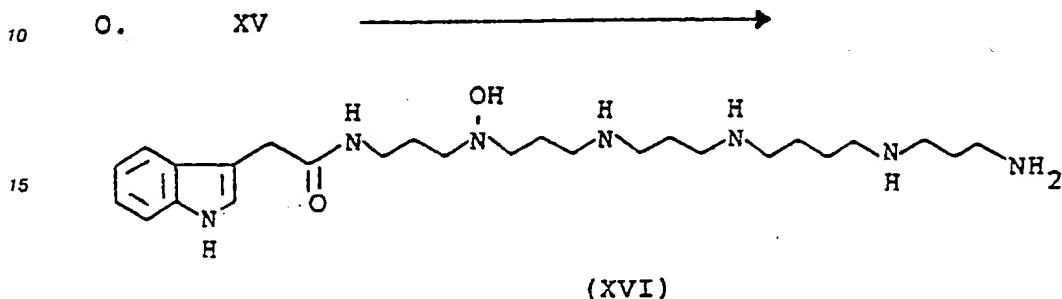


40            Under nitrogen atmosphere, 0.34 g (0.35 mmoles) of the compound of formula XIV, prepared as described above, was combined with 30 ml acetone, stirred into solution and cooled to about 0° C. in an ice/acetone bath. Then, 0.212 g (1.05 mmoles) of 85% 3-chloroperoxybenzoic acid in 20 ml of acetone was added dropwise over 8-10 minutes. The reaction was stirred for 30 minutes then diluted out to 150 ml with diethylether and washed with 2 x 25 ml 10%  $K_2CO_3$ , 2 x 25 ml  $H_2O$ , 2 x 25 ml 10%  $K_2CO_3$ , 2 x 25 ml  $H_2O$  and dried over  $K_2CO_3$ . The mixture was concentrated, chased with 2 x 30 ml dichloromethane and concentrated in vacuo to a steady weight of 0.33 g. NMR analysis showed the product to be a mixture of the desired product of formula XV, starting material and by-product. The product mixture was added to 3 ml of acetic acid to which was added 20 mg  $NaCNBH_3$ . The mixture was stirred overnight, stripped of acetic acid in vacuo, dissolved in 50 ml dichloromethane and washed 1 x 75 ml with aqueous buffer, pH 7. If necessary, the pH was adjusted to pH 7 with 1N NaOH and the dichloromethane layer was separated and washed with 1 x 50 ml with aqueous buffer, pH 7 and 1 x 25 ml brine, dried over  $Na_2SO_4$ , and concentrated

45            in vacuo to a steady weight of 0.31 g. The resulting product was chromatographed on 100 g silica gel using 95:5 ethyl acetate/methanol to elute and collecting 25 ml fractions. The purified product of formula XV came off in cuts 18-25. Those cuts were combined, concentrated, chased with 2 x 5 ml of dichloromethane and concentrated in vacuo to a white foam of 68 mg which was stored at -80° C. Cuts 26-40 which also contained the desired product also contained some impurity. Cuts 26-40 were combined, concentrated,

chased with dichloromethane and concentrated in vacuo to a steady weight of 66 mg. The latter product was combined with 6 mg of the former product which had been used for NMR studies and with a mixture which had been prepared according to the procedure described above. The resulting mixture was chromatographed on 75 g silica gel using 9:5 ethyl acetate/methanol to elute and collecting 25 ml fractions.

5 Purified product of formula XV was present in cuts 16-21. Those cuts were combined, concentrated, chased with 2 x 5 ml dichloromethane and concentrated in vacuo to a steady weight of 46 mg which was stored at -80° C.

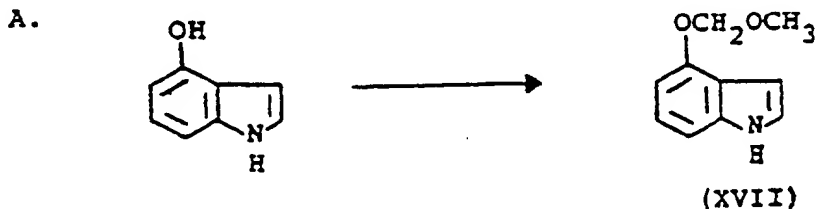


20 Under nitrogen atmosphere, 108 mg (0.109 mmoles) of compound of the formula XV, prepared as described above, was dissolved in 1.5 ml dichloromethane. Then, 2 ml of trifluoroacetic acid was added and the mixture was stirred for one hour at room temperature. The reaction mixture was then concentrated to a film and 15 ml diethylether was added. The film turned to solid and, after 30 minutes of stirring, yielded white powder. The powder was filtered, washed well with diethylether, dried under nitrogen, then evacuated to a steady weight of 109 mg of the title compound of this Example.

### 30 EXAMPLE 12

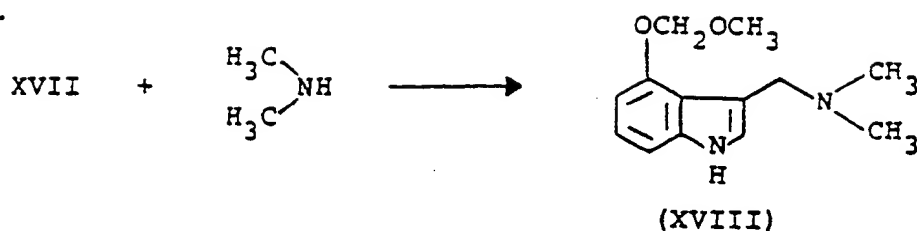
35 1H-Indole-3-acetamide, N-(20-amino-4-hydroxy-4,8,12,17-tetraazaeicos-1-yl)-4-hydroxy

Synthesis of the title compound, ascertained to be comprised by Fraction B<sub>1</sub> as described in Example 3, was accomplished as described below.



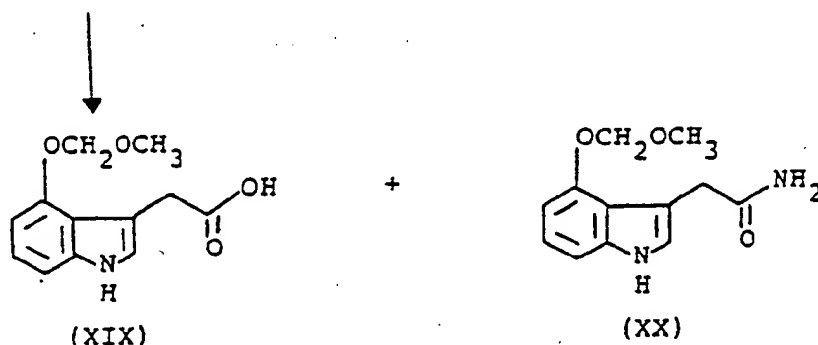
50 Under argon atmosphere using flame dried glassware, 4.0 g (30 mmoles) of 4-hydroxyindole was combined with 25 ml of Aldrich dry DMF and stirred into solution. Then, 1.44 g (30 mmoles) of NaH as a 50% oil dispersion was added. After foaming had subsided, 2.5 ml of chloromethylmethyl ether (Aldrich) was added and the resulting dark green solution was stirred overnight. Then, the solution was diluted to 125 ml with ethyl acetate, washed with 5 x 25 ml H<sub>2</sub>O and 1 x 25 ml brine, dried over Na<sub>2</sub>SO<sub>4</sub> and chromatographed using 4:1 hexane/ethyl acetate. The product containing fractions were combined and concentrated to yield 2.70 g of compound XVII as an oil.

B.



In a 50 ml, 3 neck round bottom flask equipped with a mechanical stirrer and a nitrogen atmosphere was cooled 1.3 g of aqueous formaldehyde (37%) and 2.28 g of acetic acid. Then, 3.23 ml of cooled dimethylamine (25% in H<sub>2</sub>O) was added. To the resulting cooled solution was added 2.7 g (15.2 mmoles) of compound of the formula XVII using about 1.5-2.0 ml of tetrahydrofuran as transfer solvent. The reaction was warmed to room temperature and stirred overnight. Then, 40 ml of 10% NaOH was added whereupon a precipitate formed. After stirring, the precipitate was filtered, washed with H<sub>2</sub>O and air dried to yield 3.26 g of compound of the formula XVIII.

C. XVIII



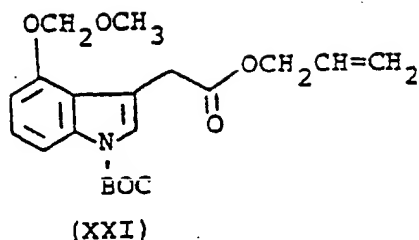
Under nitrogen atmosphere, 4.62 g of KCN was combined with 10 ml H<sub>2</sub>O and stirred into solution. Then 30 ml of ethanol and 3.26 g (14 mmoles) of compound XVIII were added and the mixture was stirred and heated to reflux for about 65 hours. The reaction was cooled and concentrated. The resulting precipitate comprising compound XX was filtered, washed with H<sub>2</sub>O and air dried. The aqueous layer was extracted with 4 x 15 ml dichloromethane, saving the organic extracts. The aqueous layer then was overlaid with 50 ml ethyl acetate and acidified to pH 2. The ethyl acetate layer was separated, degassed by bubbling nitrogen through the ethyl acetate layer, dried and concentrated to a solid which was triturated with diisopropyl ether to yield 1.03 g of compound XIX as an off-white solid. Then, all extracts from the basic aqueous layer were combined, dried, combined with the filtrates from above and concentrated to yield 0.4 g of compound XX as a solid.

D. XX → XIX

Under nitrogen atmosphere, 1.6 g of compound XX was combined with 7.5 ml of ethanol, 30 ml H<sub>2</sub>O and 1.3 g KOH. The mixture was heated to reflux and stirred overnight. Then, the reaction was cooled, extracted with 2 x 15 ml ethyl acetate, overlaid with fresh ethyl acetate and acidified to pH 2. The combined ethyl acetate layers were dried and concentrated. The resulting solid was triturated with IPE to yield 1.1 g of compound XIX.

E.

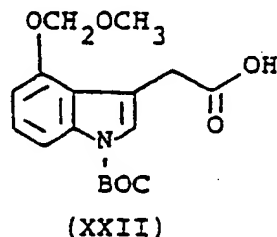
XIX



In 10 ml H<sub>2</sub>O was dissolved 1.44 g (4.25 mmoles) TBAHSO<sub>4</sub>. Then, 714 mg (8.5 mmoles) of NaHCO<sub>3</sub> was added. After foaming subsided, 1.0 g (4.25 mmoles) of compound of formula XIX was added and the mixture was stirred for one minute before 40 ml of chloroform was added. The reaction was stirred for 5 minutes, the chloroform layer was separated and then the aqueous mixture was extracted with 1 x 15 ml chloroform. The combined chloroform extracts were dried, concentrated and chased with 2 x 30 ml of acetone. Then, the product was redissolved in 30 ml of acetone under nitrogen atmosphere, 0.37 ml (4.25 mmoles) of allyl bromide was added and the reaction was stirred for 2 hours. The mixture was concentrated and chromatographed using ethyl acetate as the eluent. The product was concentrated to yield 1.1 g of an oil. All of the product was combined with 25 ml dichloromethane and 0.98 g (4.5 mmoles) of di-*t*-butyldicarbonate and 51 mg (0.425 mmoles) 4-(*N,N*-dimethylamino)pyridine were added. The reaction was stirred overnight. Then, the reaction mixture was concentrated and chromatographed using 9:1 hexane/ethyl acetate to yield, after concentration, 1.33 g of the compound of formula XXI as a viscous oil.

F.

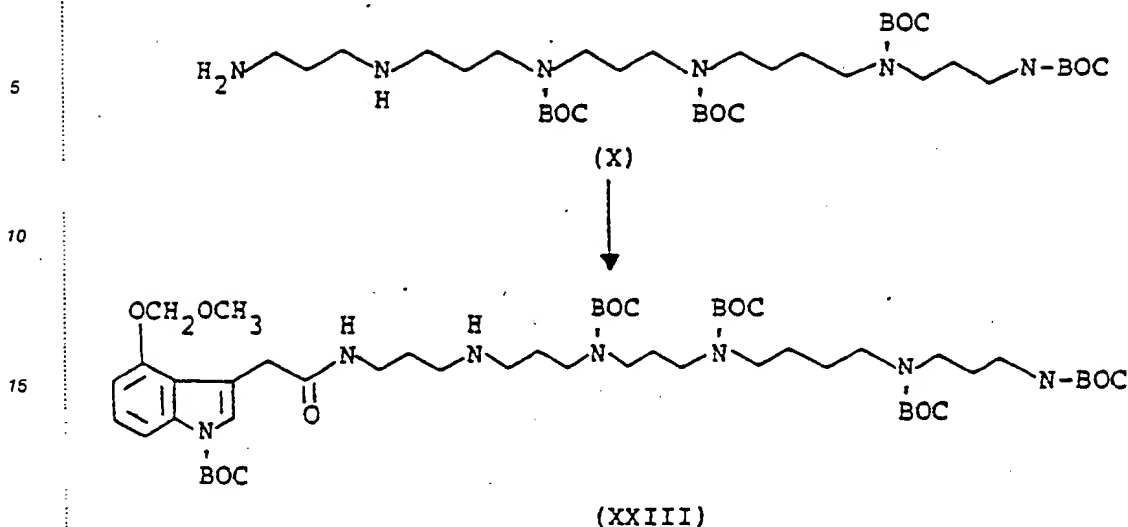
XXI



In 2 ml of methanol was dissolved 64 mg (0.17 mmoles) of compound of the formula XXI. To that solution was added 1.7 ml of 0.1 NaOH and the resulting reaction mixture was stirred overnight. The reaction then was acidified, dried, extracted with ethyl acetate and concentrated. NMR analysis showed all of the allyl ester of compound XXI had been removed but some trans esterification had occurred and some methyl ester was present. Therefore, the product was combined with the remaining amount of compound XXI (approx. 1.26 g), dissolved in 5 ml of tetrahydrofuran and cooled to 0° C. Then, 3.8 ml of 1N NaOH was added, the reaction was stirred for 5 minutes at 0° C. then allowed to warm to room temperature. The reaction resulted in two phases which required the addition of 5 ml methanol to eliminate the phases. The reaction was stirred overnight. Then, the tetrahydrofuran and methanol were stripped off and the remaining aqueous solution was overlayed with 50 ml ethyl acetate and acidified to pH 2. The ethyl acetate layer was separated, washed with 1 x 20 ml H<sub>2</sub>O, once with brine, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to a gum which crystallized when triturated with hexane to yield 1.08 g of white solid. The solid was chromatographed using 70:30 ethyl acetate/hexane and the clean product cuts were combined, concentrated and crystallized from ethyl acetate/hexane to yield 0.884 g of compound of the formula XXII.



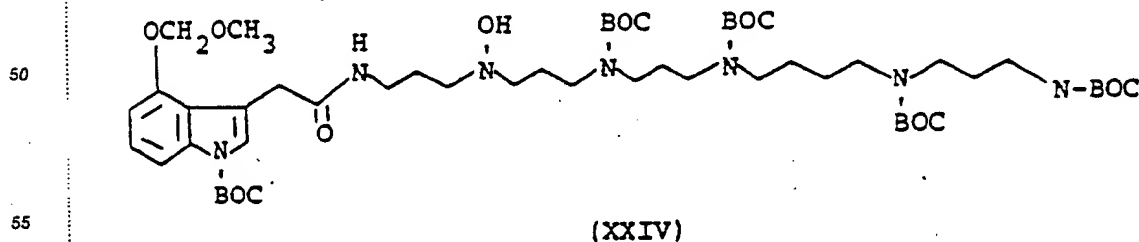
G. XXII +



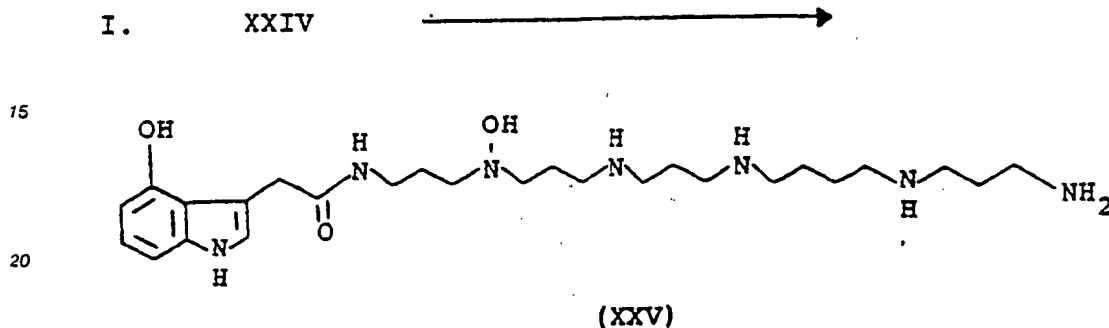
Under nitrogen atmosphere, 271 mg (0.81 mmoles) of compound of the formula XXII was dissolved with stirring in 4 ml of anhydrous tetrahydrofuran. Then, 93 mg (0.81 mmoles) of N-hydroxysuccinimide and 167 mg (0.81 mmoles) of dicyclohexylcarbodiimide were added and the mixture was stirred. After one hour and fifty minutes, 0.58 g (0.8 mmoles) of compound of the formula X, prepared according to the procedure described in Example 11, in 30 ml dichloromethane was added and the mixture stirred over the weekend. Then, the dicyclohexylurea was filtered off, and the mixture was extracted with 2 x 3 ml 1N NaOH, dried over  $K_2CO_3$  and concentrated. The product then was chromatographed on 100 g silica gel using 9:1 dichloromethane/methanol to elute everything but the desired product then with 90:10:1 dichloromethane/methanol/isopropylamine to elute the product. The product containing fractions were concentrated, chased several times with dichloromethane and stripped of solvent to yield 349 mg of compound of the formula XXIII as a white foam.

Alternatively, and preferably, a 50 ml solution containing 0.480 g (1.43 mmoles) of compound of the formula XXII in dichloromethane was treated with 0.165 g (1.43 mmoles) of N-hydroxysuccinimide followed by 0.294 g (1.43 mmoles) of dicyclohexylcarbodiimide and allowed to stir for 5 hours. The reaction mixture was filtered and the filtrate containing crude hydroxysuccinimide was added dropwise over a 20 minute period to a 100 ml solution of 1.03 g (1.43 mmoles) of compound of the formula X, prepared according to the procedure described in Example 11, in dichloromethane. The reaction was stirred for 72 hours. Then, the crude reaction mixture was washed twice with 20 ml of 1N NaOH, dried over  $K_2CO_3$ , filtered and concentrated. The product was then chromatographed on silica gel using 4:1 dichloromethane/methanol followed by 9:1:1 dichloromethane/methanol/diisopropylamine to afford 912 mg of compound of the formula XXIII.

H. XXIII



Under a nitrogen atmosphere, indoleacetamide XXIII (900 mg, 0.87 mmol) was dissolved in 40 ml of dichloromethane. To this solution was added 2-(phenylsulfonyl)-3-phenyloxaziridine (500 mg, 1.92 mmol). Progress of the reaction was monitored by thin layer chromatography. After 1 hour the reaction mixture was concentrated in vacuo to give predominantly crude nitron which was dissolved in 35 ml of acetic acid. A large excess of sodium cyanoborohydride (1.00 g, 15.9 mmol) was added and the reaction was allowed to stir for about 2 hours. The reaction was concentrated in vacuo, taken up in dichloromethane (50 ml), washed with pH 7 buffer (1 x 50 ml) adjusting the pH to 7 with 1N NaOH. The organic layer was washed again with pH 7 buffer (1 x 50 ml), dried over potassium carbonate, and concentrated in vacuo to generate crude product which was chromatographed on silica gel using 95:5 ethyl acetate/methanol to afford 612 mg (67%) of XXIV as a white foam.



By alternately pulling a vacuum and bleeding with argon three times, 3 ml of trifluoroacetic acid was degassed. Then, the flask was wrapped in aluminum foil and 98 mg of compound XXIV was added with the use of about 2.5 ml dichloromethane as a transferring agent. After 45 minutes, the mixture was concentrated to yield an oil which, upon trituration with diethyl ether, yielded 88 mg of compound XXV as a white solid which was stored at  $-80^{\circ}\text{C}$ .

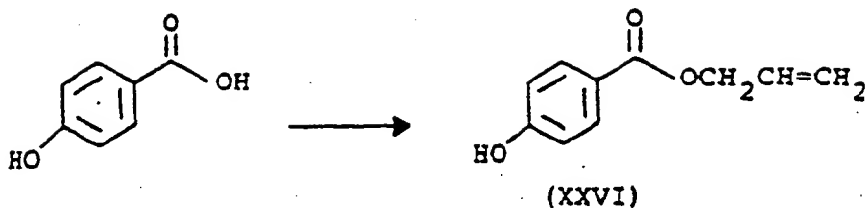
Alternatively, and preferably, to a saturated 100 ml dioxane-HCl solution, purged with argon, was added, over 1.5 minutes, 0.520 g (0.495 mmoles) of compound of formula XXIV, prepared according to the procedure described in part H, above, in 10 ml of dioxane. A precipitate formed immediately and the reaction was stirred for 1 hour. Then, diethylether was added and, after 5 minutes, the solution was filtered. The resultant solids were washed with diethylether, dried under nitrogen atmosphere and then under reduced pressure to afford 345 mg of the acid of formula XXIV' which could be stored at  $-78^{\circ}\text{C}$ . Then, to an aqueous solution, purged with argon, was added 150 mg (0.205 mmoles) of the acid of formula XXIV'. The reaction was monitored by HPLC. Upon completion of the reaction after about 7 hours, the reaction mixture was freeze dried to afford 103 mg of compound of the formula XXV as its hydrochloride salt.

### EXAMPLE 13

Benzamide, N-(20-amino-4-hydroxy-4,8,12,17-tetrazeicos-1-yl)-4-hydroxy

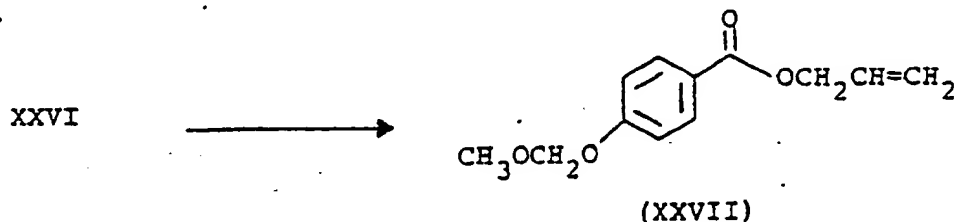
Synthesis of the title compound, ascertained to be comprised by fraction A' as described in Example 2 was accomplished as described below.

A.



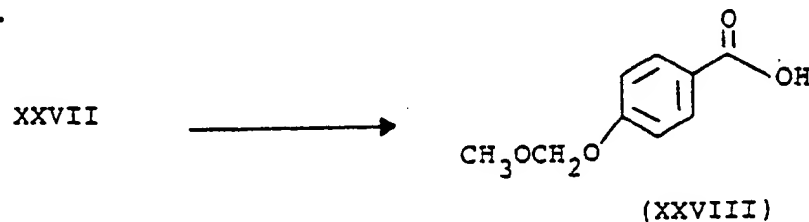
In a 400 ml beaker, 6.78 g (20 mmoles) of tetrabutyl-ammonium hydrogen sulfate was combined with 60 ml H<sub>2</sub>O and stirred into solution. Then, 3.36 g (40 mmoles) of NaHCO<sub>3</sub> was added as foaming would allow followed by the addition of 40 ml of a solution containing 2.76 g (20 mmoles) p-hydroxybenzoic acid, 0.8 g NaOH and 40 ml H<sub>2</sub>O. The reaction mixture was stirred for 5 minutes and then 200 ml of dichloromethane was added. The mixture was stirred for another 5 minutes and the layers were allowed to separate. The aqueous layer was extracted twice with 50 ml of dichloromethane. The combined dichloromethane extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, chased twice with 75 ml acetone and redissolved in 50 ml acetone. Then, 1.9 ml (22 mmoles) of allyl bromide were added and the mixture was stirred. The reaction mixture was then concentrated, taken up in 100 ml ethylacetate, washed twice with 30 ml H<sub>2</sub>O, once with 50 ml saturated bicarbonate, once with 30 ml H<sub>2</sub>O, once with brine, once with H<sub>2</sub>O and once with brine. The resulting product was dried and concentrated to yield an oil. The oil was triturated with 50 ml petroleum ether for one hour. Then, the mixture was filtered, washed well with petroleum ether and air dried to yield 1.7 g of compound of the formula XXVI, above.

B.



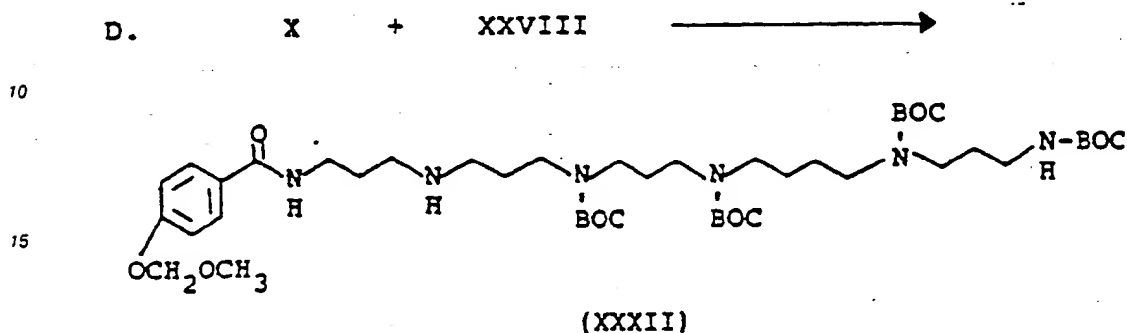
Under a nitrogen atmosphere, 1.7 g (9.55 mmoles) of compound of the formula XXVI, prepared as described in part A, above, was combined with 50 ml of Aldrich dry DMF and stirred into solution. Then, 0.38 g (9.55 mmoles) of NaH as a 60% oil dispersion was added and the reaction was stirred overnight. Then, 0.76 ml of chloromethylmethyl ether (Aldrich) was added whereupon a precipitate formed immediately. The reaction mixture was stirred for 18 hours. The reaction was quenched in 200 ml ethylacetate/100 ml H<sub>2</sub>O. The ethylacetate layer was separated, washed 3 times with 50 ml H<sub>2</sub>O, once with 50 ml 1N NaOH, once with 50 ml H<sub>2</sub>O and once with brine. The resulting solution was dried, concentrated and chromatographed on silica gel using 4:1 hexane/ethylacetate to yield 1.63 g of compound of the formula XXVII, above.

C.

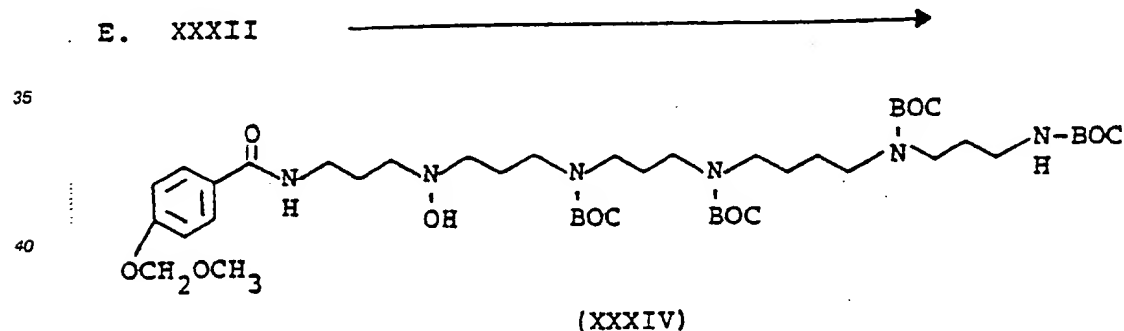


In 75 ml tetrahydrofuran was dissolved 1.62 g (7.3 mmoles) of compound of the formula XXVII, prepared as described in part B, above. Then a solution containing 0.4 g (10 mmoles) of NaOH in 15 ml of H<sub>2</sub>O was added resulting in the formation of two layers. Methanol was added until a homogeneous mixture

was obtained. The reaction was then stirred overnight. The tetrahydrofuran and methanol were stripped off in vacuo and the remaining aqueous solution was extracted twice with 15 ml ethylacetate. Then, the aqueous layer was overlaid with 50 ml of fresh ethylacetate and acidified to pH 2.5 with 6N HCl. Then ethylacetate layer was separated, washed once with 10 ml H<sub>2</sub>O and once with 25 ml brine. The resulting solution was concentrated to yield solids which were triturated with hexane, filtered and air dried to yield 1.2 g of compound of formula XXVIII, above.

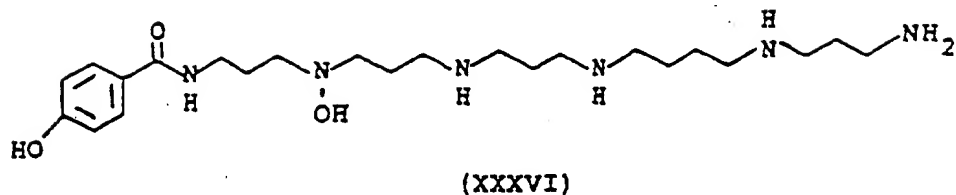


A 20 ml dichloromethane solution containing 0.127 g (0.7 mmoles) of compound of the formula XXVIII, prepared as described in Part C, above, was treated with 0.081 g (0.7 mmoles) of N-hydroxysuccinimide followed by 0.144 g (0.7 mmoles) of dicyclohexylcarbodiimide and allowed to stir for 4 hours. Then, the reaction mixture was filtered and the filtrate containing crude hydroxysuccinimide was added dropwise over a 20 minute period to a 80 ml dichloromethane solution containing 0.50 g (0.7 mmoles) of compound of the formula X, prepared according to the procedure described in Example 11, Parts A-I, above. The reaction was then stirred for hours. The reaction mixture was then washed with 1N NaOH (2 x 20 ml), dried over potassium carbonate, filtered and concentrated. The concentrate was chromatographed on silica gel using 9:1 dichloromethane/methanol followed by 9:1:0.25 dichloromethane/methanol/diisopropylamine to yield 370 mg of compound of the formula XXXII, above.



Under a nitrogen atmosphere, 0.335 g (0.38 mmoles) of compound of the formula XXXII, prepared according to the procedure described in Part D, above, was dissolved in 30 ml of dichloromethane. To this solution was added 0.230 g (0.88 mmoles) of 2-(sulfonylphenyl)-3-phenyl-oxaziridine. After 0.5 hour the reaction was concentrated in vacuo and the concentrate was dissolved in 15 ml of acetic acid. A large excess of cyanoborohydride (100 g, 1.6 mmoles) was added and the reaction was stirred for 2 hours. Then, the reaction was concentrated in vacuo, taken up in 50 ml of dichloromethane, washed once with 50 ml of pH 7 buffer, adjusting the pH to 7 with 1N NaOH. The organic layer was washed again with 50 ml of pH 7 buffer, dried over potassium carbonate and concentrated in vacuo. The concentrate was chromatographed on silica gel using 50:50 acetone/hexane to yield 253 mg of compound of the formula XXXIV, above.

F. (XXXIV)



15 Under an argon atmosphere at room temperature were combined 0.20 g (0.223 mmoles) of compound of the formula XXXIV, prepared according to the procedure described in Part E, above, and 5 ml of trifluoroacetic acid. The reaction was stirred for 1.5 hours. An additional 2 ml of trifluoroacetic was added to wash the walls of the flask. The reaction was allowed to proceed for an additional 1.5 hours. Then, the reaction was concentrated in vacuo, triturated with ethyl ether, filtered and dried under a nitrogen atmosphere to afford 218 mg of compound of the formula XXXVI, the title compound of this Example.

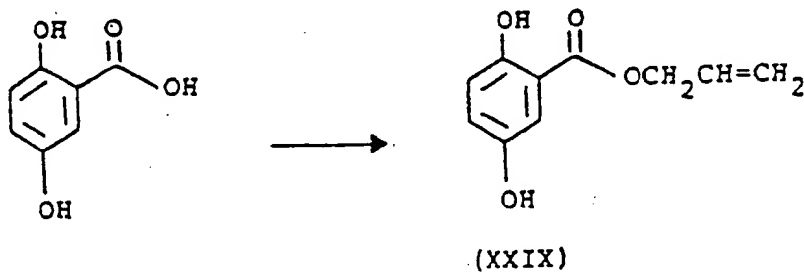
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EXAMPLE 14

25 Benzamide, N-(20-amino-4-hydroxy-4,8,12,17-tetraazaeicos-1-yl)-2,5-dihydroxy

30 Synthesis of the title compound, ascertained to be comprised by fraction A<sub>1</sub> as described in Example 3, was accomplished as described below.

A.

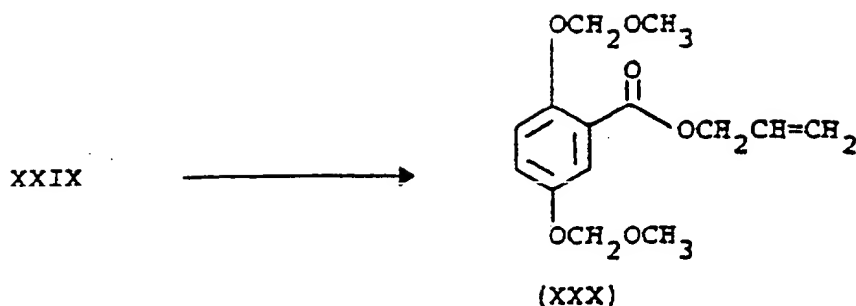


45 Employing the procedure described in Example 12, Part A, but starting with 3.08 g (20 mmoles) of 2,5-dihydroxybenzoic acid yielded 3.0 g of compound of the formula XXIX, above.

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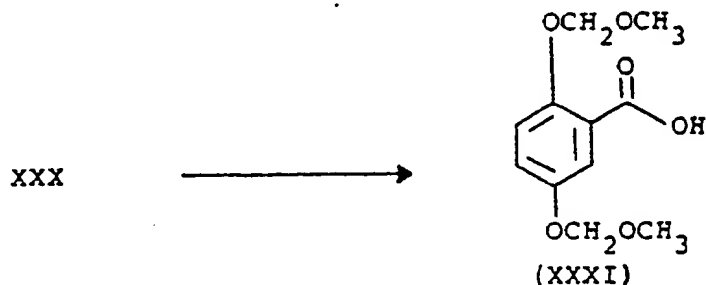
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B.



Under nitrogen atmosphere, 3.0 g (15.5 mmoles) of compound of the formula XXIX was combined with 50 ml of Aldrich dry DMF and stirred into solution. Then, 1.24 g (31 mmoles) of NaH as a 60% oil dispersion was added and the mixture was stirred. After 3 hours, 2.5 ml (33 mmoles) of chloromethylmethyl ether was added and the reaction was stirred for 18 hours. The reaction mixture was then added to 300 ml ethylacetate and 100 ml H<sub>2</sub>O. The ethylacetate layer was then separated and washed 3 times with 75 ml H<sub>2</sub>O, twice with 75 ml 1N NaOH, twice with 50 ml H<sub>2</sub>O and once with brine. The resulting solution was dried and concentrated to yield 2.9 g of an oil which was chromatographed on silica gel using 4:1 hexane/ethylacetate to yield two fractions, the second fraction of which contained 1.16 g of compound of the formula XXX, above.

C.



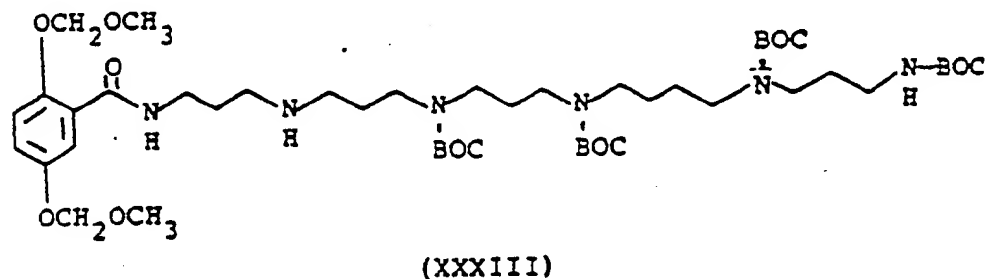
To 10 ml of tetrahydrofuran was added 1.16 g (4.1 mmoles) of compound of the formula XXX, above. Then, 0.24 g (6 mmoles) of NaOH in 10 ml H<sub>2</sub>O was added which resulted in the formation of two phases. Methanol was added to the mixture until one phase was obtained and then the mixture was stirred for 18 hours. The solvents were then removed and the remaining aqueous solution was extracted twice with 15 ml of ethylacetate. The aqueous layer was then overlaid with 35 ml of fresh ethylacetate and the pH was adjusted to 2.5 with 6N HCl. The ethylacetate layer was then separated, washed once with 10 ml H<sub>2</sub>O, and once with brine, dried and concentrated to yield 1.0 g of compound of the formula XXXI, above, as an oil.

D.

X

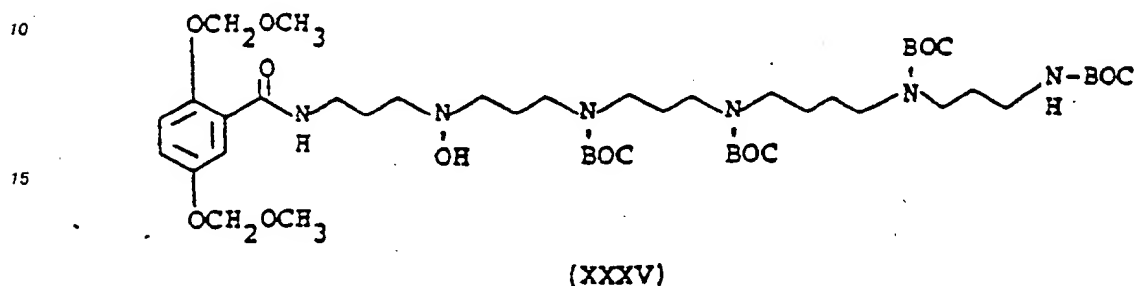
+

XXXI



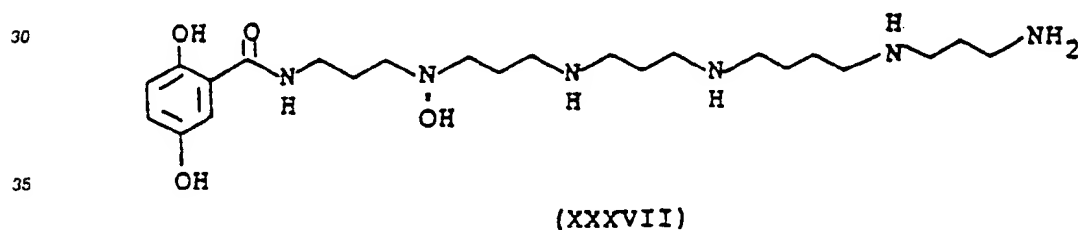
Employing the procedure described in Example 13, Part D, and using 0.17 g (0.7 mmoles) of compound of the formula XXXI, prepared as described in Part C, above, 0.081 g (0.7 mmoles) N-hydroxysuccinimide, 0.144 g (0.7 mmoles) dicyclohexylcarbodiimide and 0.50 g (0.7 mmoles) of compound of the formula X, prepared according to the procedure described in Example 11, Parts A-I, yielded, after chromatography, 370 mg of compound of the formula XXXIII, above.

E. XXXIII



Employing the procedure described in Example 13, Part E, and using 0.308 g (0.33 mmoles) of compound of the formula XXXIII, prepared according to the procedure described in Part D, above, and 0.250 g (0.96 mmoles) of 2-(sulfonylphenyl)-3-phenyl-oxaziridine yielded, after silica gel chromatography using ethylacetate followed by 50:50 acetone/hexane, 210 mg of compound of the formula XXXV, above.

F. XXXV

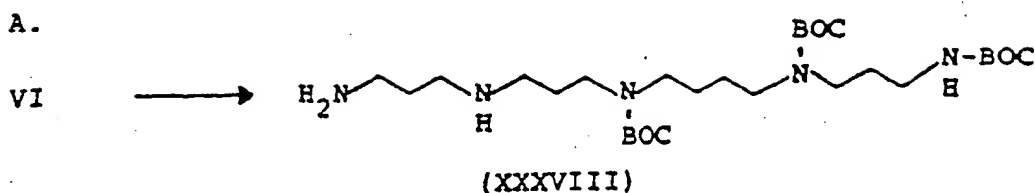


Employing the procedure described in Example 13, Part F, and using 0.100 g (0.104 mmoles) of compound of the formula XXXV, prepared according to the procedure described in Part E, above, yielded 108 mg of compound of the formula XXXVII, the title compound of this Example.

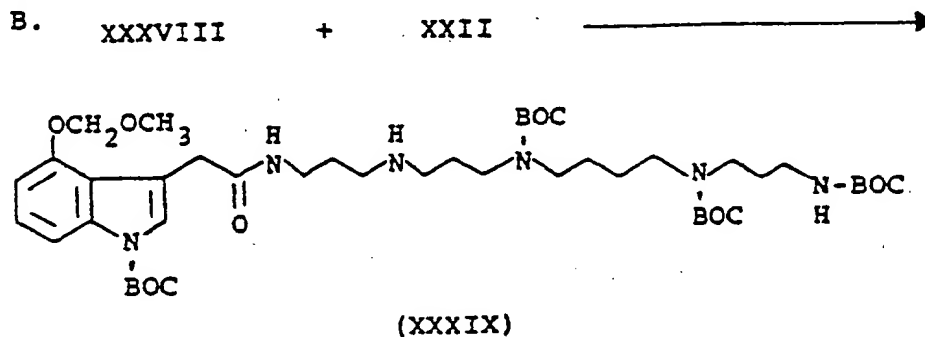
#### EXAMPLE 15

1H-Indole-3-acetamide, N-(16-amino-4-hydroxy-4,8,13-triazahexadec-1-yl)-4-hydroxy

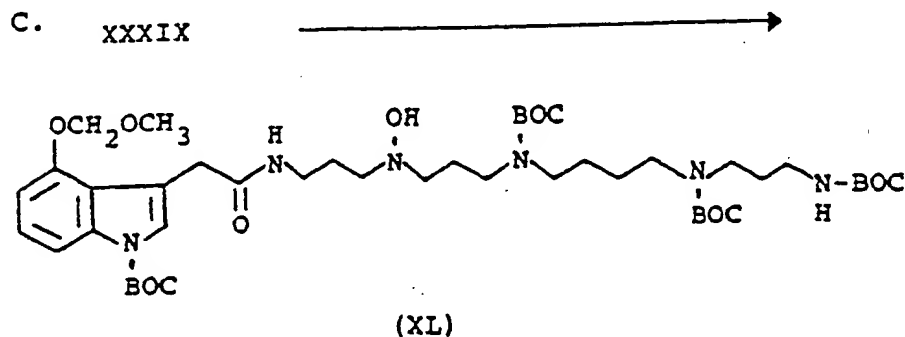
Synthesis of the title compound, ascertained to be comprised by fraction A<sub>2</sub> as described in Example 3, was accomplished as described below.



Employing the procedure described in Example 11, Part D, and using 1.15 g (2.07 mmoles) of compound of the formula VI, prepared as described in Example 11, Parts A-E, yielded 1.10 g of crude compound of the formula XXXVIII, above.



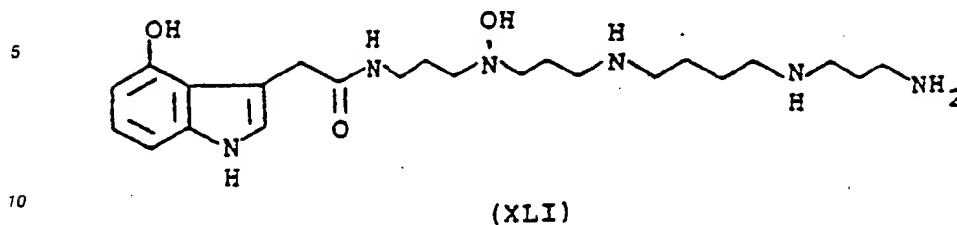
Employing the alternate procedure described in Example 12, Part G, and using a dichloromethane solution (15 ml) containing 0.228 g (6.8 mmoles) of compound of the formula XXII, prepared as described in Example 12, Parts A to F, above, 0.380 g (6.8 mmoles) of compound of the formula XXXVIII, prepared as described in Part A, above, 0.078 g (6.8 mmoles) of N-hydroxysuccinimide and 0.140 g (6.8 mmoles) of dicyclohexylcarbodiimide afforded 0.407 g of compound of the formula XXXIX after silica gel chromatography using 9:1 dichloromethane/methanol followed by 9:1:0.5 dichloromethane/methanol/diisopropylamine.



Employing the procedure described in Example 12, Part H, and using 0.350 g (0.4 mmoles) of compound of the formula XXXIX and 0.230 g (0.88 mmoles) of 2-(sulfonylphenyl)-3-phenyloxaziridine gave 0.274 g of compound of the formula XL, above, after silica gel chromatography using 50:50 acetone/hexane. The product contained a minor amount of phenylsulfonamide and therefore was further purified on silica gel using ethylacetate followed by 50:50 acetone/hexane.

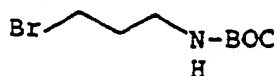


D. XL



15 Employing the alternate procedure of Example 12, Part I, and using 0.166 g (0.186 mmoles) of compound of the formula XL, prepared according to the procedure described in Part C, above, provided after dioxane/HCl treatment crude acid. The acid of formula XL was dissolved in water for 8 hours, followed by freeze drying to yield 88 mg of compound of the formula XLI.

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PREPARATION A

Under nitrogen atmosphere, 34.5 g (157.6 mmoles) of 3-bromopropylamine·HBr in 600 ml of N,N-dimethylformamide was stirred. To that solution was added 34.4 g (157.6 mmoles) of di-t-butylidicarbonate followed by 32.3 ml (236 mmoles) triethylamine. A precipitate formed immediately. The reaction was stirred overnight. The reaction mixture was then diluted to 1.5 liters with ethylacetate, washed once with 500 ml of 1N HCl, three times with 500 ml water, once with brine and dried over Na<sub>2</sub>SO<sub>4</sub>. After concentration, the product was chromatographed on 800 g silica gel using 4:1 hexane/ethylacetate and the fractions were monitored by thin layer chromatography (KMNO<sub>4</sub>/I<sub>2</sub>). The fractions containing the product were combined, concentrated in vacuo, chased twice with 50 ml dichloromethane and purged with high vacuum to yield 25.8 g of the product of this Preparation.

## Claims

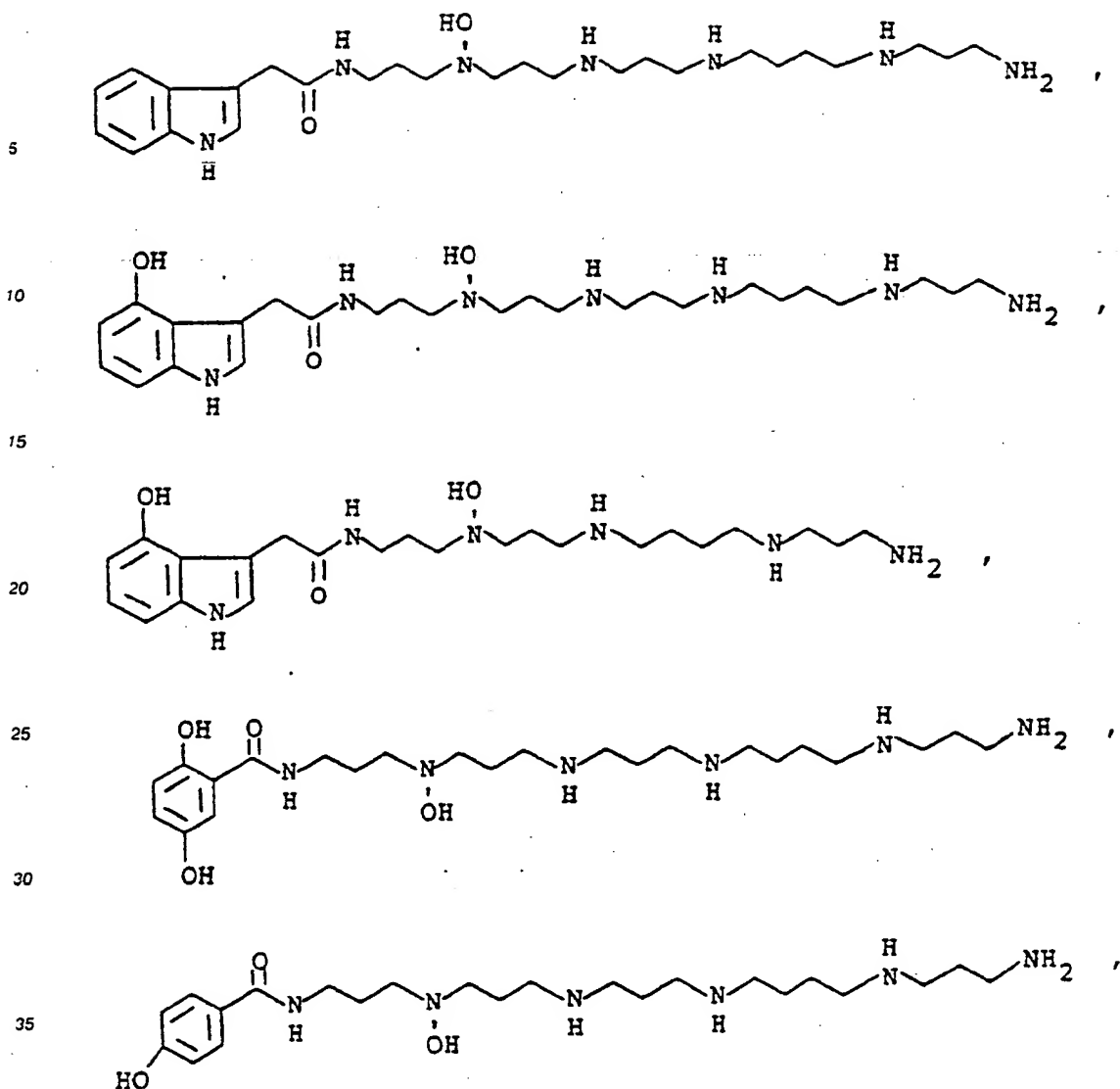
40

1. A substantially pure compound selected from the group consisting of

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50

55



40 a compound having the following identifying characteristics:

- (a) present in a fraction from crude venom of the *Agelenopsis aperta* spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min. and a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 22.75 minutes;
- 45 (b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min. and a solvent system using a non-linear gradient program of 0% → 0% B, 100% → 100% A [0 → 5 min.], then 0% → 10% B, 100% → 90% A [5 → 20 min.] (Waters curve 1) then 10% → 20% B, 90% → 80% A [20 → 30 min.] (Waters curve 6) then 20% → 50% B, 80% → 50% A [30 → 40 min.] (Waters curve 11), where A is 0.1% aqueous
- 50 TFA and B is acetonitrile, at about 21.5 minutes; and
- (c) FAB MS: high resolution: 505.3861, and the pharmaceutically-acceptable salts thereof.

2. A substantially pure polypeptide selected from the group consisting of a polypeptide having the following identifying characteristics:

- 55 (a) present in a fraction from crude venom of the *Agelenopsis aperta* spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min. and a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 38 minutes;

- (b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 10 ml/min. and a solvent system using a non-linear gradient program of 20% → 30% B, 80% → 70% A [0 → 40 min.] (Waters curve 6), where A is 0.1% aqueous TFA and B is acetonitrile, at about 22 minutes;
- 5 (c) an amino-terminal amino acid sequence comprising:  
 $\text{H}_2\text{N}$ -glu-lys-gly-leu-pro-glu-gly-ala-glu-cys-asp-gly-asn-glu-ser-asp-cys-lys-cys-ala-gly-gln-trp-ile-lys-cys-arg-cys-pro-trp-lys-trp-his-ile-thr-gly-glu-gly-pro-cys-thr-cys-glu-arg-gly-leu-lys-lys-thr-cys-ile-ser-lys-leu-ser-asp-pro-asn-arg-asn-glu-trp-leu-ser-; and  
 (d) FAB MS: 7267;
- 10 and a polypeptide having substantially the same amino acid sequence and substantially the same calcium blocking activity as said polypeptide,  
 a polypeptide of the formula  
 $\text{asp-cys-val-gly-glu-ser-gln-gln-cys-ala-asp-trp-ala-gly-pro-his-cys-cys-asp-gly-tyr-tyr-cys-thr-cys-arg-tyr-phe-pro-lys-cys-ile-cys-val-asn-asn-asn-CONH}_2$
- 15 and a polypeptide having substantially the same amino acid sequence and substantially the same calcium channel blocking activity as said polypeptide,  
 a polypeptide having the following identifying characteristics:  
 (a) present in a fraction from crude venom of the *Agelenopsis aperta* spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 39 minutes;
- 20 (b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 10 ml/min. and a solvent system using a non-linear gradient program of 20% → 25% B, 80% → 75% A [0 → 30 min.] (Waters curve 6) then 25% → 50% B, 75% → 50% A [30 → 45 min.] (Waters curve 11), where A is 0.1% aqueous TFA and B is acetonitrile, at about 36 minutes; and
- 25 (c) an amino-terminal amino acid sequence comprising:  
 $\text{H}_2\text{N}$ -ala-lys-ala-leu-pro-pro-gly-ser-val-cys-asp-gly-asn-glu-ser-asp-cys-lys-cys-tyr-gly-lys-trp-his-lys-cys-arg-cys-pro-trp-lys-trp-his-phe-thr-gly-glu-gly-pro-cys-thr-cys-glu-lys-gly-met-lys-his-thr-cys-ile-thr-lys-leu-his-cys-pro-asn-lys-ala-glu-trp-gly-leu-asp-trp-; and
- 30 (d) Ion-Spray MS: 7793;  
 and a polypeptide having substantially the same amino acid sequence and substantially the same calcium channel blocking activity as said polypeptide,  
 a polypeptide of the formula
- 35  $\text{H}_2\text{N}$ -asp-glu-pro-cys-ile-pro-leu-gly-lys-ser-cys-ser-trp-lys-ile-gly-thr-pro-tyr-cys-cys-pro-his-pro-asp-asp-ala-gly-arg-arg-thr-trp-cys-leu-val-asp-tyr-ser-arg-phe-val-thr-ile-cys-ser-gly-arg-lys-tyr-CONH<sub>2</sub>  
 and a polypeptide having substantially the same amino acid sequence and substantially the same calcium channel blocking activity as said polypeptide,  
 a polypeptide having the following identifying characteristics:
- 40 (a) present in a fraction from crude venom of the *Agelenopsis aperta* spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 43 minutes;
- 45 (b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 10 mm x 250 mm, 300 Å pore size, 5 µ particle size column using a flow rate of 3.5 ml/min. and a solvent system using a linear gradient program of 25% → 40% B, 75% → 60% A [0 → 30 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 20.25 minutes;
- (c) an amino-terminal amino acid sequence comprising:  
 $\text{H}_2\text{N}$ -ile-val-gly-gly-lys-thr-ala-lys-phe-gly-asp-tyr-pro-trp-met-val-ser-ile-glu-glu-lys-asn-lys-lys-gly-gly-phe-asp-; and
- 50 (d) a molecular weight of about 20,000;  
 and a polypeptide having substantially the same amino acid sequence and substantially the same calcium channel blocking activity as said polypeptide,  
 a polypeptide having the following identifying characteristics:
- 55 (a) present in a fraction from crude venom of the *Agelenopsis aperta* spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 43 minutes;

and

- (b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 10 mm x 250 mm, 300 Å pore size, 5 µ particle size column using a flow rate of 3.5 ml/min. and a solvent system using a linear gradient program of 25% → 40% B, 75% → 60% A [0 → 30 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 22.5 minutes;

and a polypeptide having substantially the same amino acid sequence and substantially the same calcium channel blocking activity as said polypeptide,

a polypeptide having the following identifying characteristics:

- (a) present in a fraction from crude venom of the Agelenopsis aperta spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 48.3 minutes;

(b) an amino-terminal amino acid sequence comprising:

H<sub>2</sub>N-glu-ala-thr-glu-ala-ala-lys-val-leu-ser-asn-leu-asp-glu-thr-val-asp-pro-; and

- (c) a molecular weight of about 80,000

and a polypeptide having substantially the same amino acid sequence and substantially the same calcium channel blocking activity as said polypeptide, and the pharmaceutically-acceptable salts thereof.

3. A substantially pure polypeptide according to claim 2 selected from the group consisting of a polypeptide having the following identifying characteristics:

- (a) present in a fraction from crude venom of the Agelenopsis aperta spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min. and a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 38 minutes;

- (b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 10 ml/min. and a solvent system using a non-linear gradient program of 20% → 30% B, 80% → 70% A [0 → 40 min.] (Waters curve 6), where A is 0.1% aqueous TFA and B is acetonitrile, at about 22 minutes;

(c) an amino-terminal amino acid sequence comprising:

H<sub>2</sub>N-glu-lys-gly-leu-pro-glu-gly-ala-glu-cys-asp-gly-asn-glu-ser-asp-cys-lys-cys-ala-gly-gln-trp-ile-lys-cys-arg-cys-pro-trp-lys-trp-his-ile-thr-gly-glu-gly-pro-cys-thr-cys-glu-arg-gly-leu-lys-lys-thr-cys-ile-ser-lys-leu-ser-asp-pro-asn-arg-asn-glu-trp-leu-ser-; and

(d) FAB MS: 7267,

a polypeptide of the formula

asp-cys-val-gly-glu-ser-gln-gln-cys-ala-asp-trp-ala-gly-pro-his-cys-cys-asp-gly-tyr-tyr-cys-thr-cys-arg-tyr-phe-pro-lys-cys-ile-cys-val-asn-asn-asn-CONH<sub>2</sub>,

- a polypeptide having the following identifying characteristics:

- (a) present in a fraction from crude venom of the Agelenopsis aperta spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 39 minutes;

- (b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 10 ml/min. and a solvent system using a non-linear gradient program of 20% → 25% B, 80% → 75% A [0 → 30 min.] (Waters curve 6) then 25% → 50% B, 75% → 50% A [30 → 45 min.] (Waters curve 11), where A is 0.1% aqueous TFA and B is acetonitrile, at about 36 minutes; and

(c) an amino-terminal amino acid sequence comprising:

H<sub>2</sub>N-ala-lys-ala-leu-pro-gly-ser-val-cys-asp-gly-asn-glu-ser-asp-cys-lys-cys-tyr-gly-lys-trp-his-lys-cys-arg-cys-pro-trp-lys-trp-his-phe-thr-gly-glu-gly-pro-cys-thr-cys-glu-lys-gly-met-lys-his-thr-cys-ile-thr-lys-leu-his-cys-pro-asn-lys-ala-glu-trp-gly-leu-asp-trp-; and

- (d) Ion-Spray MS: 7793,

a polypeptide of the formula

H<sub>2</sub>N-asp-glu-pro-cys-ile-pro-leu-gly-lys-ser-cys-ser-trp-lys-ile-gly-thr-pro-tyr-cys-cys-pro-his-pro-asp-asp-ala-gly-arg-arg-thr-trp-cys-leu-val-asp-tyr-ser-arg-phe-val-thr-ile-cys-ser-gly-arg-lys-tyr-CONH<sub>2</sub>,

a polypeptide having the following identifying characteristics:

- (a) present in a fraction from crude venom of the Agelenopsis aperta spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 43 minutes;

(b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 10 mm x 250 mm, 300 Å pore size, 5 µ particle size column using a flow rate of 3.5 ml/min. and a solvent system using a linear gradient program of 25% → 40% B, 75% → 60% A [0 → 30 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 20.25 minutes;

(c) an amino-terminal amino acid sequence comprising:

H<sub>2</sub>N-ile-val-gly-gly-lys-thr-ala-lys-phe-gly-asp-tyr-pro-trp-met-val-ser-ile-glu-glu-lys-asn-lys-lys-gly-gly-phe-asp-; and

(d) a molecular weight of about 20,000,

a polypeptide having the following identifying characteristics:

(a) present in a fraction from crude venom of the *Agelenopsis aperta* spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 43 minutes; and

(b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 10 mm x 250 mm, 300 Å pore size, 5 µ particle size column using a flow rate of 3.5 ml/min. and a solvent system using a linear gradient program of 25% → 40% B, 75% → 60% A [0 → 30 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 22.5 minutes,

a polypeptide having the following identifying characteristics:

(a) present in a fraction from crude venom of the *Agelenopsis aperta* spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 48.3 minutes;

(b) an amino-terminal amino acid sequence comprising:

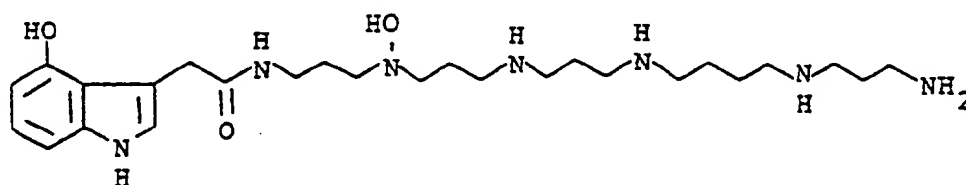
H<sub>2</sub>N-glu-ala-thr-glu-ala-ala-lys-val-leu-ser-asn-leu-asp-glu-thr-val-asp-pro-; and

(c) a molecular weight of about 80,000,

and the pharmaceutically-acceptable salts thereof.

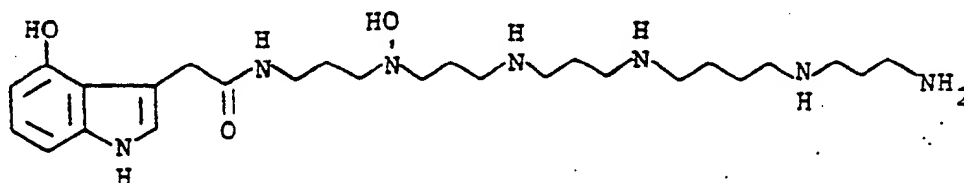
4. A pharmaceutical composition for antagonizing excitatory amino acid neurotransmitters which affect a cell in the nervous system of a mammal, said composition comprising an excitatory amino acid neurotransmitter antagonizing amount of a compound according to claim 1 or a pharmaceutically-acceptable salt thereof and a pharmaceutically-acceptable diluent or carrier.

5. A pharmaceutical composition for blocking calcium channels in a cell in the nervous system of a mammal which comprises a calcium channel blocking amount of a compound according to claim 1 of the formula



or a pharmaceutically-acceptable salt thereof and a pharmaceutically-acceptable diluent or carrier.

6. A pharmaceutical composition for antagonizing excitatory amino acid neurotransmitters which affect a cell in the nervous system of a mammal and blocking calcium channels in a cell in the nervous system of a mammal, said composition comprising an excitatory amino acid neurotransmitter antagonizing and calcium channel blocking amount of a compound according to claim 1 of the formula



or a pharmaceutically-acceptable salt thereof and a pharmaceutically-acceptable diluent or carrier.

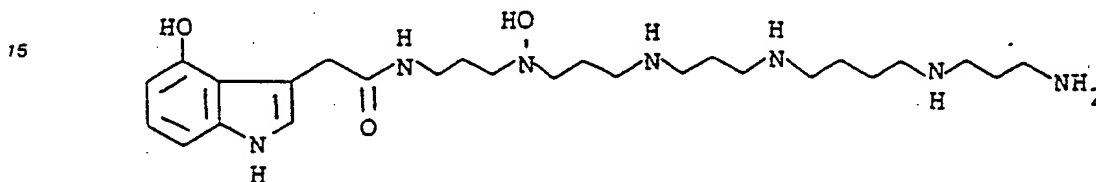
7. A pharmaceutical composition for blocking calcium channels in a cell of a mammal which comprises a calcium channel blocking amount of a polypeptide according to claim 2 or a pharmaceutically-acceptable salt thereof and a pharmaceutically-acceptable diluent or carrier.

8. A pharmaceutical composition for blocking calcium channels in a cell of a mammal which comprises a calcium channel blocking amount of a polypeptide according to claim 3 or a pharmaceutically-acceptable salt thereof and a pharmaceutically-acceptable diluent or carrier.

9. A compound as claimed in any one of claims 1 to 3 or a pharmaceutically acceptable salt thereof for use as a medicament

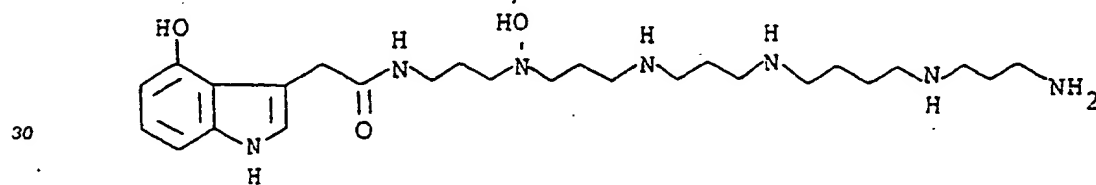
10. The use of a compound as claimed in claim 1 or of a pharmaceutically acceptable salt thereof for the manufacture of a medicament for antagonizing excitatory amino acid neurotransmitters which affect a cell in the nervous system of a mammal.

11. The use of a compound according to claim 1 of the formula



or of a pharmaceutically-acceptable salt thereof for blocking calcium channels in a cell in the nervous system of a mammal.

12. The use of a compound according to claim 1 of the formula



or of a pharmaceutically-acceptable salt thereof for antagonizing excitatory amino acid neurotransmitters which affect a cell in the nervous system of a mammal and blocking calcium channels in a cell in the nervous system of a mammal.

13. The use of a polypeptide according to claim 2 or 3 or of a pharmaceutically-acceptable salt thereof for blocking calcium channels in a cell of a mammal.

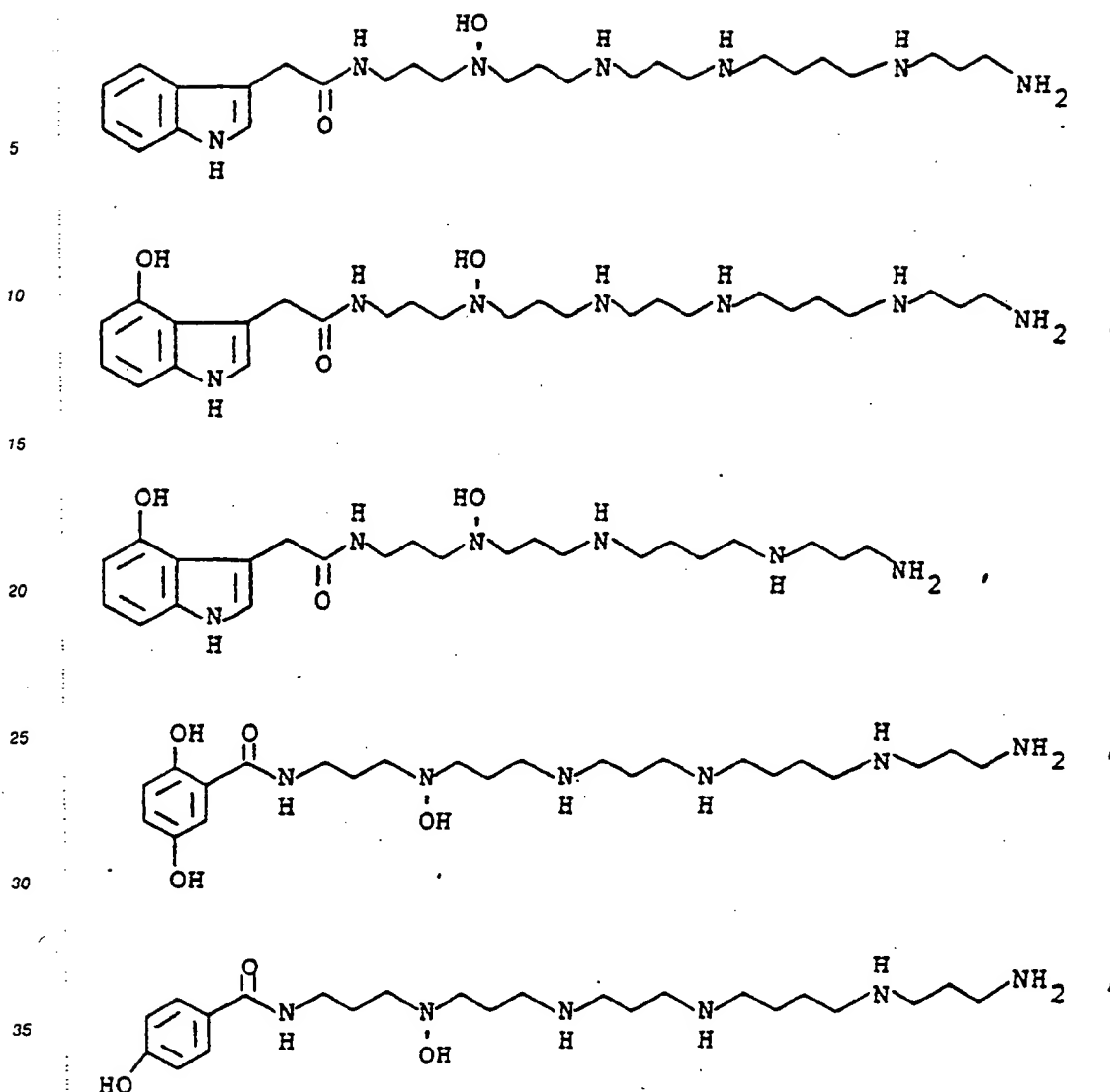
40 Claims for the following Contracting States: ES, GR

1. A process for preparing a substantially pure compound selected from the group consisting of

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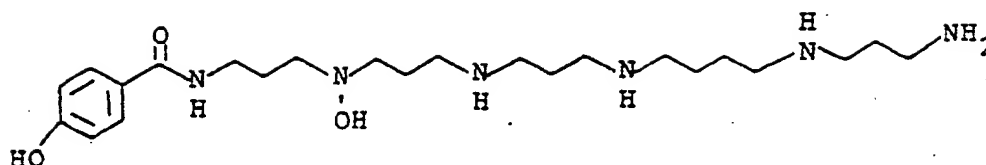


40 a compound having the following identifying characteristics:

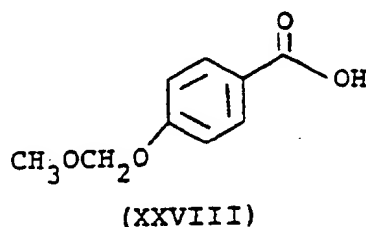
- (a) present in a fraction from crude venom of the *Agelenopsis aperta* spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min. and a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 22.75 minutes;
- 45 (b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min. and a solvent system using a non-linear gradient program of 0% → 0% B, 100% → 100% A [0 → 5 min.], then 0% → 10% B, 100% → 90% A [5 → 20 min.] (Waters curve 1) then 10% → 20% B, 90% → 80% A [20 → 30 min.] (Waters curve 6) then 20% → 50% B, 80% → 50% A [30 → 40 min.] (Waters curve 11), where A is 0.1% aqueous
- 50 TFA and B is acetonitrile, at about 21.5 minutes; and
- (c) FAB MS: high resolution: 505.3861, and the pharmaceutically-acceptable salts thereof, characterized in that:

(a) when a compound of the formula

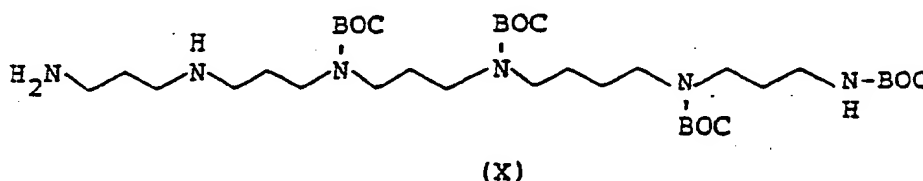
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is desired, whole venom of *Agelenopsis aperta* is eluted from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 μ particle size) using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 10% acetonitrile, 95% → 90% 0.1% aqueous trifluoroacetic acid and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 12.5 minutes is collected and, optionally, said fraction is lyophilized, resuspended in water and lyophilized from water or, alternatively, a compound of the formula

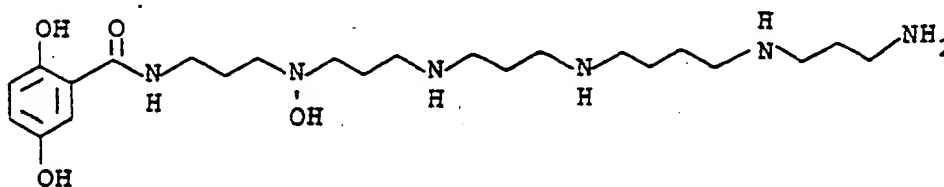


in a reaction inert solvent is reacted under an inert atmosphere with N-hydroxysuccinimide followed by dicyclohexylcarbodiimide and then a compound of the formula



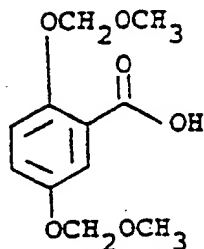
in a reaction inert solvent, the product is then reacted under an inert atmosphere with 2-(sulfonylphenyl)-3-phenyl-oxaziridine followed by cyanoborohydride and the product thereof is reacted under an inert atmosphere with trifluoroacetic acid;

(b) when a compound of the formula



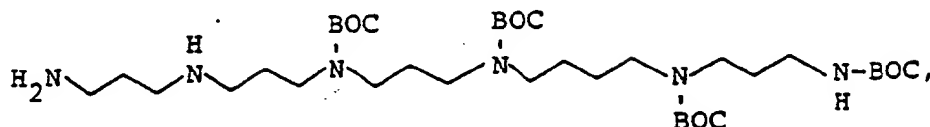
is desired, whole venom of *Agelenopsis aperta* is eluted from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 μ particle size) using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% acetonitrile, 95% → 80% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] then 20% → 70% acetonitrile, 80% → 30% 0.1% aqueous trifluoroacetic acid [30 → 55 min.] and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 21 minutes is collected and loaded onto a C-4 Vydac® column (10 mm x 250 mm, 300 Å pore size, 5 μ particle size) and is eluted using a flow rate of 4.0 ml/min., a solvent system using a linear gradient program of 0% → 10% acetonitrile, 100% → 90% 0.1% aqueous trifluoroacetic acid and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 7 minutes is collected and, optionally, said fraction is lyophilized, resuspended in water and lyophilized, or, alternatively, a compound of the formula





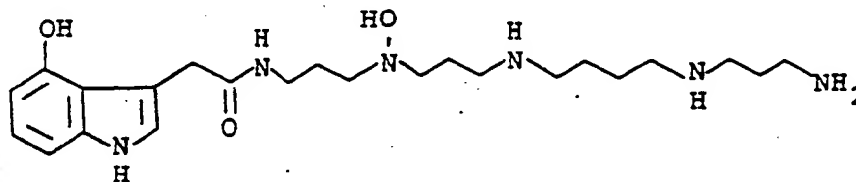
(XXXI)

in a reaction inert solvent is reacted under an inert atmosphere with N-hydroxysuccinimide followed by dicyclohexylcarbodiimide and then a compound of the formula

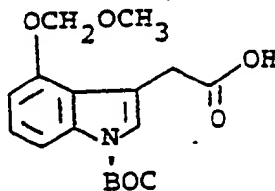


(X)

the product is then reacted under an inert atmosphere with 2-(sulfonylphenyl)-3-phenyl-oxaziridine followed by cyanoborohydride and the product thereof is reacted under an inert atmosphere with trifluoroacetic acid; (c) when a compound of the formula



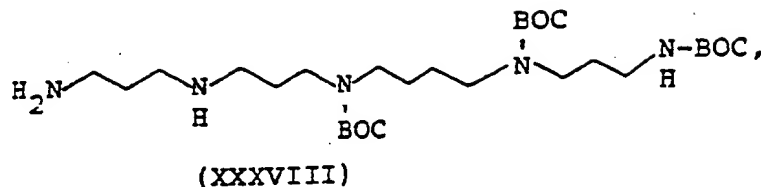
is desired, whole venom of *Agelenopsis aperta* is eluted from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 μ particle size) using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% acetonitrile, 95% → 80% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] then 20% → 70% acetonitrile, 80% → 30% 0.1% aqueous trifluoroacetic acid [30 → 55 min.] and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 21 minutes is collected and loaded onto a C-4 Vydac® column (10 mm x 250 mm, 300 Å pore size, 5 μ particle size) and is eluted using a flow rate of 4.0 ml/min., a solvent system using a linear gradient program of 0% → 10% acetonitrile, 100% → 90% 0.1% aqueous trifluoroacetic acid and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 9 minutes is collected and, optionally, said fraction is lyophilized, resuspended in water and lyophilized, or, alternatively, a compound of the formula



(XXII)

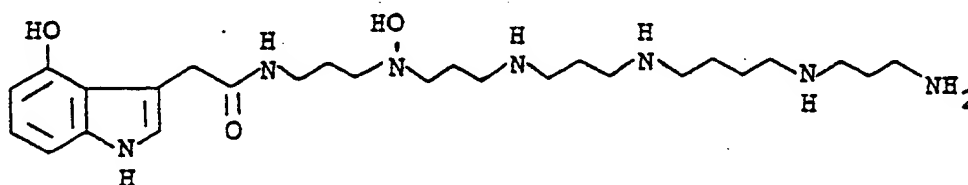
in a reaction inert solvent is reacted under an inert atmosphere with N-hydroxysuccinimide followed by

dicyclohexylcarbodiimide and then a compound of the formula

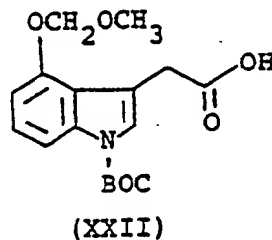


10 the product is then reacted under an inert atmosphere with 2-(sulfonylphenyl)-3-phenyl-oxaziridine followed by cyanoborohydride and the product thereof is either reacted with trifluoroacetic acid under an inert atmosphere or, under an inert atmosphere, dissolved in saturated dioxane-HCl, recovered therefrom and added to an aqueous solution under an inert atmosphere;

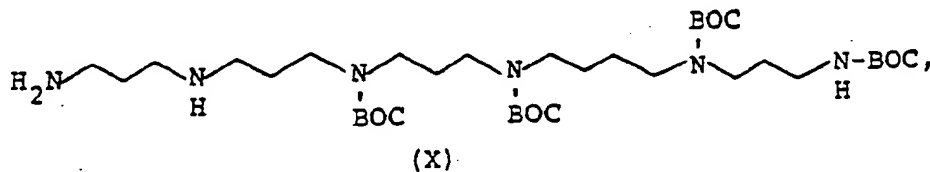
15 (d) when a compound of the formula



25 is desired, whole venom of *Agelenopsis aperta* is eluted from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 μ particle size) using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% acetonitrile, 95% → 80% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] then 20% → 70% acetonitrile, 80% → 30% 0.1% aqueous trifluoroacetic acid [30 → 55 min.] and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 22.75 minutes is collected and loaded onto a C-4 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 μ particle size) and is eluted using a flow rate of 15 ml/min., a solvent system using a non-linear gradient program of 0% → 0% acetonitrile, 100% → 100% 0.1% aqueous trifluoroacetic acid [0 → 5 min.], then 0% → 10% acetonitrile, 100% → 90% 0.1% aqueous trifluoroacetic acid [5 → 20 min.] (Waters curve 1), then 10% → 20% acetonitrile, 90% → 80% 0.1% aqueous trifluoroacetic acid [20 → 30 min.] (Waters curve 6), then 20% → 50% acetonitrile, 80% → 50% 0.1% aqueous trifluoroacetic acid [30 → 40 min.] (Waters curve 11) and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 18.5 minutes is collected and, optionally, said fraction is lyophilized, resuspended in water and lyophilized, or, alternatively, a compound of the formula

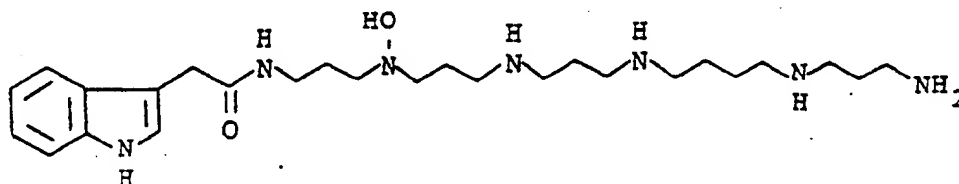


50 in a reaction inert solvent is reacted under an inert atmosphere with N-hydroxysuccinimide followed by dicyclohexylcarbodiimide and then a compound of the formula

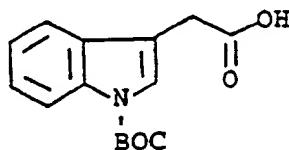


the product is then reacted under an inert atmosphere with 2-(sulfonylphenyl)-3-phenyl-oxaziridine followed by cyanoborohydride and the product thereof is either reacted with trifluoroacetic acid under an inert atmosphere or, under an inert atmosphere, dissolved in saturated dioxane-HCl, recovered therefrom and added to an aqueous solution under an inert atmosphere;

(e) when a compound of the formula

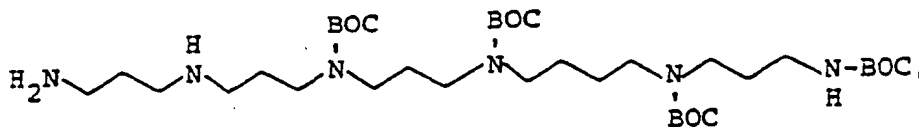


is desired, whole venom of *Agelenopsis aperta* is eluted from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 μ particle size) using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% acetonitrile, 95% → 80% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] then 20% → 70% acetonitrile, 80% → 30% 0.1% aqueous trifluoroacetic acid [30 → 55 min.] and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 27.5 minutes is collected and, optionally, said fraction is lyophilized, resuspended in water and lyophilized, or, alternatively, whole venom of *Agelenopsis aperta* is eluted from said column using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 0% → 20% acetonitrile, 100% → 80% 0.1% aqueous trifluoroacetic acid and peak detection at 220 nm, the fraction which elutes at about 26.0 minutes is collected and loaded onto a Dynamax Phenyl column (4.6 mm x 250 mm, 60 Å pore size, 8 μ particle size) and is eluted using a flow rate of 1 ml/min., isocratic conditions of 10% acetonitrile, 90% 0.1% aqueous trifluoroacetic acid and peak detection at 220 nm, the fraction which elutes at about 55.27 minutes is collected and, optionally, lyophilized, resuspended in water and lyophilized, or, alternatively, a compound of the formula



(XIII)

in a reaction inert solvent is reacted under an inert atmosphere with N-hydroxysuccinimide, dicyclohexylcarbodiimide and then a compound of the formula



(X)

the product in acetone is then reacted under an inert atmosphere with 3-chloroperoxybenzoic acid, recovered therefrom and reacted with cyanoborohydride in the presence of acetic acid and then the product thereof is reacted with trifluoroacetic acid under an inert atmosphere;

(f) when a compound is desired having the following identifying characteristics

- (a) present in a fraction from crude venom of the *Agelenopsis aperta* spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 μ particle size column using a flow rate of 15 ml/min. and a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 22.75 minutes;
- (b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 μ particle size column using a flow rate of 15 ml/min. and a solvent system

using a non-linear gradient program of 0% → 0% B, 100% → 100% A [0 → 5 min.], then 0% → 10% B, 100% → 90% A [5 → 20 min.] (Waters curve 1) then 10% → 20% B, 90% → 80% A [20 → 30 min.] (Waters curve 6) then 20% → 50% B, 80% → 50% A [30 → 40 min.] (Waters curve 11), where A is 0.1% aqueous TFA and B is acetonitrile, at about 21.5 minutes; and

- 5 (c) FAB MS: high resolution: 505.3861.  
whole venom of *Agelenopsis aperta* is eluted from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% acetonitrile, 95% → 80% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] then 20% → 70% acetonitrile, 80% → 30% 0.1% aqueous trifluoroacetic acid [30 → 55 min.] and monochromatic peak
- 10 detection at 220-230 nm, the fraction which elutes at about 22.75 minutes is collected and loaded onto a C-4 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) and is eluted using a flow rate of 15 ml/min., a solvent system using a non-linear gradient program of 0% → 0% acetonitrile, 100% → 100% 0.1% aqueous trifluoroacetic acid [0 → 5 min.], then 0% → 10% acetonitrile, 100% → 90% 0.1% aqueous trifluoroacetic acid [5 → 20 min.] (Waters curve 1), then 10% → 20% acetonitrile, 90% → 80% 0.1% aqueous trifluoroacetic acid [20 → 30 min.] (Waters curve 6), then 20% → 50% acetonitrile, 80% → 50% 0.1% aqueous trifluoroacetic acid [30 → 40 min.] (Waters curve 11) and monochromatic peak detection at
- 15 220-230 nm, the fraction which elutes at about 21.5 minutes is collected and, optionally, said fraction is lyophilized, resuspended in water and lyophilized; and optionally converting said compounds to their pharmaceutically-acceptable salts by methods known per se.
- 20 2. A process for preparing a substantially pure polypeptide selected from the group consisting of a polypeptide (I) having the following identifying characteristics:
  - (a) present in a fraction from crude venom of the *Agelenopsis aperta* spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min. and a solvent system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 38 minutes;
  - 25 (b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 10 ml/min. and a solvent system using a non-linear gradient program of 20% → 30% B, 80% → 70% A [0 → 40 min.] (Waters curve 6), where A is 0.1% aqueous TFA and B is acetonitrile, at about 22 minutes;
  - 30 (c) an amino-terminal amino acid sequence comprising:  
H<sub>2</sub>N-glu-lys-gly-leu-pro-glu-gly-ala-glu-cys-asp-gly-asn-glu-ser-asp-cys-lys-cys-ala-gly-gln-trp-ile-lys-cys-arg-cys-pro-trp-lys-trp-his-ile-thr-gly-glu-gly-pro-cys-thr-cys-glu-arg-gly-leu-lys-lys-thr-cys-ile-ser-lys-leu-ser-asp-pro-asn-arg-asn-glu-trp-leu-ser-; and
  - (d) FAB MS: 7267;
  - 35 and a polypeptide having substantially the same amino acid sequence and substantially the same calcium blocking activity as said polypeptide (I),  
a polypeptide (II) of the formula  
asp-cys-val-gly-glu-ser-gln-gln-cys-ala-asp-trp-ala-gly-pro-his-cys-cys-asp-gly-tyr-tyr-cys-thr-cys-arg-tyr-phe-pro-lys-cys-ile-cys-val-asn-asn-asn-CONH<sub>2</sub>
  - 40 and a polypeptide having substantially the same amino acid sequence and substantially the same calcium channel blocking activity as said polypeptide (II),  
a polypeptide (III) having the following identifying characteristics:
    - (a) present in a fraction from crude venom of the *Agelenopsis aperta* spider which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min., a solvent
    - 45 system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B, 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 39 minutes;
    - (b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 10 ml/min. and a solvent system using a non-linear gradient program of 20% → 25% B, 80% → 75% A [0 → 30 min.] (Waters curve 6) then
    - 50 25% → 50% B, 75% → 50% A [30 → 45 min.] (Waters curve 11), where A is 0.1% aqueous TFA and B is acetonitrile, at about 36 minutes; and
    - (c) an amino-terminal amino acid sequence comprising:  
H<sub>2</sub>N-ala-lys-ala-leu-pro-pro-gly-ser-val-cys-asp-gly-asn-glu-ser-asp-cys-lys-cys-tyr-gly-lys-trp-his-lys-cys-arg-cys-pro-trp-lys-trp-his-phe-thr-gly-glu-gly-pro-cys-thr-cys-glu-lys-gly-met-lys-his-thr-cys-ile-thr-lys-leu-
    - 55 his-cys-pro-asn-lys-ala-glu-trp-gly-leu-asp-trp-; and
    - (d) Ion-Spray MS: 7793;
- and a polypeptide having substantially the same amino acid sequence and substantially the same calcium channel blocking activity as said polypeptide (III),

a polypeptide (IV) of the formula

$\text{H}_2\text{N}-\text{asp}-\text{glu}-\text{pro}-\text{cys}-\text{ile}-\text{pro}-\text{leu}-\text{gly}-\text{lys}-\text{ser}-\text{cys}-\text{ser}-\text{trp}-\text{lys}-\text{ile}-\text{gly}-\text{thr}-\text{pro}-\text{tyr}-\text{cys}-\text{cys}-\text{pro}-\text{his}-\text{pro}-\text{asp}-\text{asp}-\text{ala}-\text{gly}-\text{arg}-\text{arg}-\text{thr}-\text{trp}-\text{cys}-\text{leu}-\text{val}-\text{asp}-\text{tyr}-\text{ser}-\text{arg}-\text{phe}-\text{val}-\text{thr}-\text{ile}-\text{cys}-\text{ser}-\text{gly}-\text{arg}-\text{lys}-\text{tyr}-\text{CONH}_2$

and a polypeptide having substantially the same amino acid sequence and substantially the same calcium

5 channel blocking activity as said polypeptide (IV),

a polypeptide (V) having the following identifying characteristics:

(a) present in a fraction from crude venom of the Agelenopsis aperta spider which elutes off a C-18 Vydac®  
22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min., a solvent  
system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B,  
10 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 43 minutes;

(b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 10 mm x  
250 mm, 300 Å pore size, 5 µ particle size column using a flow rate of 3.5 ml/min. and a solvent system  
using a linear gradient program of 25% → 40% B, 75% → 60% A [0 → 30 min.], where A is 0.1% aqueous  
TFA and B is acetonitrile, at about 20.25 minutes;

15 (c) an amino-terminal amino acid sequence comprising:

$\text{H}_2\text{N}-\text{ile}-\text{val}-\text{gly}-\text{gly}-\text{lys}-\text{thr}-\text{ala}-\text{lys}-\text{phe}-\text{gly}-\text{asp}-\text{tyr}-\text{pro}-\text{trp}-\text{met}-\text{val}-\text{ser}-\text{ile}-\text{glu}-\text{glu}-\text{lys}-\text{asn}-\text{lys}-\text{lys}-\text{gly}-\text{gly}-\text{phe}-\text{asp}$ ; and

(d) a molecular weight of about 20,000;

and a polypeptide having substantially the same amino acid sequence and substantially the same calcium

20 channel blocking activity as said polypeptide (V),

a polypeptide (VI) having the following identifying characteristics:

(a) present in a fraction from crude venom of the Agelenopsis aperta spider which elutes off a C-18 Vydac®  
22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min., a solvent  
system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B,  
25 80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 43 minutes;  
and

(b) present in a fraction of the fraction described in (a), above, which elutes off a C-18 Vydac® 10 mm x  
250 mm, 300 Å pore size, 5 µ particle size column using a flow rate of 3.5 ml/min. and a solvent system  
using a linear gradient program of 25% → 40% B, 75% → 60% A [0 → 30 min.], where A is 0.1% aqueous  
30 TFA and B is acetonitrile, at about 22.5 minutes;

and a polypeptide having substantially the same amino acid sequence and substantially the same calcium  
channel blocking activity as said polypeptide (VI),

a polypeptide (VII) having the following identifying characteristics:

(a) present in a fraction from crude venom of the Agelenopsis aperta spider which elutes off a C-18 Vydac®  
35 22 mm x 250 mm, 300 Å pore size, 10 µ particle size column using a flow rate of 15 ml/min., a solvent  
system using a linear gradient program of 5% → 20% B, 95% → 80% A [0 → 30 min.] then 20% → 70% B,  
80% → 30% A [30 → 55 min.], where A is 0.1% aqueous TFA and B is acetonitrile, at about 48.3 minutes;

(b) an amino-terminal amino acid sequence comprising:

$\text{H}_2\text{N}-\text{glu}-\text{ala}-\text{thr}-\text{glu}-\text{ala}-\text{ala}-\text{lys}-\text{val}-\text{leu}-\text{ser}-\text{asn}-\text{leu}-\text{asp}-\text{glu}-\text{thr}-\text{val}-\text{asp}-\text{pro}$ ; and

40 (c) a molecular weight of about 80,000

and a polypeptide having substantially the same amino acid sequence and substantially the same calcium  
channel blocking activity as said polypeptide (VII), and the pharmaceutically-acceptable salts thereof,  
characterized in that

(a) when substantially pure polypeptide (I) is desired, whole venom of Agelenopsis aperta is eluted  
45 from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) using a flow rate of 15  
ml/min., a solvent system using a linear gradient program of 5% → 20% acetonitrile, 95% → 80% 0.1%  
aqueous trifluoroacetic acid [0 → 30 min.] then 20% → 70% acetonitrile, 80% → 30% 0.1% aqueous  
trifluoroacetic acid [30 → 55 min.] and monochromatic peak detection at 220-230 nm, the fraction which  
elutes at about 38 minutes is collected and loaded onto a C-18 Vydac® column (22 mm x 250 mm, 300 Å  
50 pore size, 10 µ particle size) and is eluted using a flow rate of 10 ml/min., a solvent system using a non-  
linear gradient program of 20% → 30% acetonitrile, 80% → 70% 0.1% aqueous trifluoroacetic acid [0 → 40  
min.] (Waters curve 6) and peak detection at 220-230 nm, the fraction which elutes at about 22 minutes is  
collected and, optionally, lyophilized, resuspended in water and lyophilized;

(b) when substantially pure polypeptide (II) is desired, whole venom of Agelenopsis aperta is eluted  
55 from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) using a flow rate of 15  
ml/min., a solvent system using a linear gradient program of 5% → 20% acetonitrile, 95% → 80% 0.1%  
aqueous trifluoroacetic acid [0 → 30 min.] then 20% → 70% acetonitrile, 80% → 30% 0.1% aqueous  
trifluoroacetic acid [30 → 55 min.] and monochromatic peak detection at 220-230 nm, the fraction which

elutes at about 40 minutes is collected and loaded onto a C-4 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) and is eluted using a flow rate of 10 ml/min., a solvent system using a non-linear gradient program of 20% → 30% acetonitrile, 80% → 70% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] (Waters curve 6) then 30% → 50% acetonitrile, 70% → 50% 0.1% aqueous trifluoroacetic acid [30 → 45 min.] (Waters curve 11) and peak detection at 220-230 nm, the fraction which elutes at about 22 minutes is collected and, optionally, lyophilized, resuspended in water and lyophilized;

(c) when substantially pure polypeptide (III) is desired, whole venom of Agelenopsis aperta is eluted from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% acetonitrile, 95% → 80% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] then 20% → 70% acetonitrile, 80% → 30% 0.1% aqueous trifluoroacetic acid [30 → 55 min.] and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 39 minutes is collected and loaded onto a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) and is eluted using a flow rate of 10 ml/min., a solvent system using a non-linear gradient program of 20% → 25% acetonitrile, 80% → 75% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] (Waters curve 6) then 25% → 50% acetonitrile, 75% → 50% 0.1% aqueous trifluoroacetic acid [30 → 45 min.] (Waters curve 11) and peak detection at 220-230 nm, the fraction which elutes at about 36 minutes is collected and, optionally, lyophilized, resuspended in water and lyophilized;

(d) when substantially pure polypeptide (IV) is desired, whole venom of Agelenopsis aperta is eluted from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% acetonitrile, 95% → 80% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] then 20% → 70% acetonitrile, 80% → 30% 0.1% aqueous trifluoroacetic acid [30 → 55 min.] and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 40 minutes is collected and loaded onto a C-4 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) and is eluted using a flow rate of 10 ml/min., a solvent system using a non-linear gradient program of 20% → 30% acetonitrile, 80% → 70% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] (Waters curve 6) then 30% → 50% acetonitrile, 70% → 50% 0.1% aqueous trifluoroacetic acid [30 → 45 min.] (Waters curve 11) and peak detection at 220-230 nm, the fraction which elutes at about 27.5 minutes is collected and, optionally, lyophilized, resuspended in water and lyophilized;

(e) when substantially pure polypeptide (V) is desired, whole venom of Agelenopsis aperta is eluted from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% acetonitrile, 95% → 80% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] then 20% → 70% acetonitrile, 80% → 30% 0.1% aqueous trifluoroacetic acid [30 → 55 min.] and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 43 minutes is collected and loaded onto a C-18 Vydac® column (10 mm x 250 mm, 300 Å pore size, 5 µ particle size) and is eluted using a flow rate of 3.5 ml/min., a solvent system using a linear gradient program of 25% → 40% acetonitrile, 75% → 60% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] and peak detection at 220-230 nm, the fraction which elutes at about 20.25 minutes is collected and, optionally, lyophilized, resuspended in water and lyophilized;

(f) when substantially pure polypeptide (VI) is desired, whole venom of Agelenopsis aperta is eluted from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% acetonitrile, 95% → 80% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] then 20% → 70% acetonitrile, 80% → 30% 0.1% aqueous trifluoroacetic acid [30 → 55 min.] and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 43 minutes is collected and loaded onto a C-18 Vydac® column (10 mm x 250 mm, 300 Å pore size, 5 µ particle size) and is eluted using a flow rate of 3.5 ml/min., a solvent system using a linear gradient program of 25% → 40% acetonitrile, 75% → 60% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] and peak detection at 220-230 nm, the fraction which elutes at about 22.5 minutes is collected and, optionally, lyophilized, resuspended in water and lyophilized;

(g) when substantially pure polypeptide (VII) is desired, whole venom of Agelenopsis aperta is eluted from a C-18 Vydac® column (22 mm x 250 mm, 300 Å pore size, 10 µ particle size) using a flow rate of 15 ml/min., a solvent system using a linear gradient program of 5% → 20% acetonitrile, 95% → 80% 0.1% aqueous trifluoroacetic acid [0 → 30 min.] then 20% → 70% acetonitrile, 80% → 30% 0.1% aqueous trifluoroacetic acid [30 → 55 min.] and monochromatic peak detection at 220-230 nm, the fraction which elutes at about 48.3 minutes is collected and optionally, lyophilized, resuspended in water and lyophilized;

or, alternatively, any of said polypeptides is synthesized by methods known per se; and when polypeptides having substantially the same amino acid sequence as any of said polypeptides are desired, such polypeptide or polypeptides are synthesized by methods known per se; and optionally converting said polypeptides to the pharmaceutically-acceptable salts thereof by methods known per se.



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**Polyamines and polypeptides useful as antagonists of excitatory amino acid neurotransmitters and/or as blockers of calcium channels.**

This invention relates to certain polyamines and polypeptides found to be present in the venom of the *Agelenopsis aperta* spider. The polyamines of this invention and the salts thereof antagonize excitatory amino acid neurotransmitters, which neurotransmitters affect cells of various organisms and are useful in antagonizing said neurotransmitters, per se, in the treatment of excitatory amino acid neurotransmitter mediated diseases and conditions and in the control of invertebrate pests. The polypeptides of this invention and one of said polyamines and the salts thereof block calcium channels in cells of various organisms and are useful in blocking said calcium channels in cells, per se, in the treatment of calcium channel mediated diseases and conditions and in the control of invertebrate pests. This invention also relates to compositions

comprising said polyamines, polypeptides and salts thereof.



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## EUROPEAN SEARCH REPORT

Application Number  
EP 90304397  
- page 1 -

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	TETRAHEDRON LETTERS vol. 28, no. 30, 1987, pages 3509,3510, GB; T. TESHIMA et al.: "Synthesis of a new neurotoxin NSTX-3 of papua new guinean spider" * the whole document *	1,4	C07D209/18 C07C235/50 C07C235/60 C07K15/08 C07K7/10 A61K31/165 A61K31/405 A61K37/02
A	TETRAHEDRON LETTERS vol. 28, no. 30, 1987, pages 3511-3514, GB; Y. HASHIMOTO et al.: "Synthesis of spider toxin (JSTX-3) and its analogs" * the whole document *	1,4	
A	CHEMICAL ABSTRACTS vol. 108, no. 3, 18 January 1988, page 189, abstract no. 17508t, Columbus, Ohio, US; H. PAN-HOU et al.: "Inhibitory effect of 2,4-dihydroxyphenylacetylas- paragine, a common moiety of spider toxin, on glutamate binding to rat brain synaptic membranes" & Neurosci. Lett. 1987, vol. 81, nos. 1/2, pages 199-203 * abstract *	1,4	
A	CHEMICAL ABSTRACTS vol. 108, no. 3, 18 January 1988, page 189, abstract no. 17509u, Columbus, Ohio, US; M.E. ADAMS et al.: "Structures and biological activities of three sy- naptic antagonists from orb weaver spider venom" & Biochem. Biophys. Res. Commun. 1987, vol. 148, no. 2, pages 678-683 * abstract *	1,4	
The present search report has been drawn up for all claims		1,4-6, 10-12	
Place of search BERLIN		Date of completion of the search 23.07.1991	Examiner KYRIAKAKOU, G.
CATEGORY OF CITED DOCUMENTS		I : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : technological background O : non-written disclosure P : intermediate document	

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## CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claims:
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

## X LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims 1,4-6,10-12  
essentially poly amino components
2. claims 2,3,7-9  
essentially polypeptide components

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims: 1,4-6,10-12



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## EUROPEAN SEARCH REPORT

Application number

EP 90304397

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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	CHEMICAL ABSTRACTS vol. 108, no. 25, 20 June 1988, page 185, abstract no. 217532h, Columbus, Ohio, US; T. BUDD et al.: "Isolation and character- ization of glutamate receptor antagonists from venoms of orb-web spiders" & Brain Res. 1988, vol. 448, no. 1, pages 30-39, * abstract *	1,4	
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P,A	WO-A-9002114 (NATIONAL RESEARCH DEVELOP- MENT CORPORATION) * pages 1-4, line 5 *	1,4	
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